

STUDIES ON
GASTRIC ACHYLIA
WITH
SPECIAL REGARD TO NEUTRAL
RED EXCRETION

By

LARS HALLÉN

Published in
Upsala Läkareförenings Förhandlingar
(Acta Societatis Medicorum Upsaliensis)
Vol. LIV: 3-4, 1949

STUDIES ON GASTRIC ACHYLIA WITH SPECIAL REGARD TO NEUTRAL RED EXCRETION

INAUGURAL DISSERTATION

BY

LARS HALLÉN

MED. LIC., UPPL.

By due permission of the most experienced Medical
Faculty of the University of Uppsala, to be publicly
discussed in the institution of Pathology, on May
13th 1949, at 10:00 a. m., for the degree of doctor
of medicine

UPPSALA 1949

ALMQVIST & WIKSELLS BOKTRYCKERI AB

300/
50



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41550014_0008

From the Department of Theoretical Medicine (Head: Professor JAN WALDENSTRÖM, M. D.) and the State Institute of Human Genetics and Race Biology (Head: Professor GUNNAR DAHLBERG, M. D., LL. D.).



Studies on Gastric Achylia with Special Regard to Neutral Red Excretion.

By

LARS HALLÉN.

Preface.....	178
Chapter I. <i>Survey of the literature</i>	179
The primary acidity and its regulation.....	179
Gastric excretion of neutral red.....	183
Pepsin.....	189
Aminopolypeptidase and intrinsic factor.....	191
Chapter II. <i>Methods</i>	196
Aspiration technique.....	196
Determination of acidity.....	199
Determination of chloride.....	200
Determination of neutral red.....	201
Determination of pepsin.....	201
Determination of aminopolypeptidase.....	204
Chapter III. <i>Material</i>	205
Chapter IV. <i>Neutral Red Tests</i>	206
In cases of hypochylia.....	207
In cases of pernicious anaemia.....	209
In cases of simple achylia.....	210
Excretion in acid instillates of varying strength.....	210
Discussion of neutral red excretion.....	210
Excretion in cases of tuberculosis.....	213
Calculation of primary acidity.....	216
Sources of error.....	218
Occurrence of dye excretion among cases of histamine refractory achylia....	220
Observations on gastric mucosa, removed at operations.....	223
Chapter V. <i>Experiments with acid instillation</i>	223
In normal cases.....	224
In hypochlorhydria.....	231
In achlorhydria.....	233
Instillation of NH_4NO_3	237

Gastric secretion after histamine combined with atropine and pilocarpine....	237
Determination of glucosamine.....	240
Chapter VI. <i>Pepsin in cases of achylia</i>	242
In simple achylia.....	243
In pernicious anaemia.....	244
In glycine and different acid instillates.....	246
The connection between pepsin and acid secretion.....	247
Hydrochloric acid therapy and peptic effect in achylia.....	248
Chapter VII. <i>Aminopolypeptidase</i>	249
In pernicious anaemia.....	250
In simple achylia.....	250
In gastric mucosa.....	252
Chapter VIII. <i>Discussion</i>	254
Chapter IX. <i>Summary</i>	259
Clinical data	262
References.....	265

Preface.

This investigation was started in 1943. Its original purpose was to study cases of achylia with regard to severity and especially to make an effort to differentiate the achylia of pernicious anaemia from other cases with histamine refractory achylia. To that end the excretion of neutral red has been examined together with the secretion of pepsin and aminopolypeptidase. In connection with the neutral red tests some experiments with acid instillations were made and the question of primary acidity and its reduction was touched upon.

The material was collected in the Medical Clinic of the University Hospital in Upsala, for the main part when Professor Gustaf Bergmark was chief of the Clinic. It is my pleasant duty to thank him for his interest and help in my work.

During the whole investigation Professor Jan Waldenström of all others has supported me. His advice and encouragement has been of the very greatest value and I wish to thank him for this and for his friendship.

For discussions concerning many fundamental problems my thanks are due to Professor Torsten Teorell. Partly these experiments start from his own investigations. With his great experience he always helped me in a generous manner.

My warm thanks are also due to Professor Gunnar Dahlberg who has devoted much time to discuss the problems and analyse the results. His critical judgment and positive attitude have been of great importance to me.

I am greatly indebted to Professor Gunnar Ågren for his kind help with the determination of aminopolypeptidase. I also wish to thank him for his personal interest and for many profitable discussions.

Many thanks are also due to Professors G. Blix, E. Hedvall, O. Hultén, H. Laurell and M. Wrethé for valuable help in different connections.

The investigation was partly performed in the Central Laboratory, where Laborator Anders Grönwall has helped me in every way. I thank him cordially for his kind interest.

For the most part the practical performance of intubation etc. and the chemical analyses have been carried out by Mrs Maj-Britt Sandberg, laboratory nurse. She performed the most laborious part of the work and I wish to thank her for valuable help.

My thanks are also due to Dr. Elisabeth Palmer, London, who translated the paper rapidly and carefully.

The work has been carried out with the help of grants from the Foundation "Thérèse och Johan Anderssons Minne", the Regnell Foundation, and the Swedish State Funds for Promoting Medical Research.

CHAPTER I.

Survey of the Literature.

The Primary Acidity and its Regulation.

It may be said that there are in the literature two ways of looking at these problems:

1. PAVLOV's, who maintains that the acid is secreted by the parietal cells in a concentration of constant strength but is later neutralized by the mucus;

2. ROSEMAN's, who considers that the acid is first secreted in varying strength but with constant chloride content. Hypo- and anacidity respectively are explained by the fact that to an increasing extent secretion of neutral chloride takes place instead of acid. ROSEMAN's theory of the constant chloride content has not stood the test of subsequent criticism, which has shown a connection between acidity and the total amount of chlorides. — Among other authors who maintain that acid can be produced in varying strength we may mention KATSCH & KALK (1926), McLEAN & GRIFFITHS (1928).

One may say that PAVLOV's theory of a constant primary acidity has on the whole established itself even if several of its adherents have modified it in one way or another. The difficulty has been, however to explain how this primary acidity is reduced to the level at which it is found in examinations of the gastric juice.

It has proved very difficult to determine primary acidity. HOLLANDER (1932) calculated it as 167 mN and LIU, YUAN & LIM (1934) as 176 mN. TEORELL (1940) has directly worked out primary acidity with the help

of a glycine buffer instillate in the cat's stomach and determined a primary acidity of 208 ± 6 mN, but reckons with the possibility that there are other alkalibinding substances than HCl and glycine-HCl in this test and that therefore his figure is a little too high. — Primary acidity is usually regarded as about 170 mN. IHRE accepts this figure, ELLIOT, RISHOLM & ÖBRINK (1942) work with 175 mN.

In an investigation on dog and cat LINDE, TEORELL & ÖBRINK (1947) adopted a modification of Teorell's technique with glycine instillate by using Sørensen's formaldehyde titration to determine the glycine content of the instillate. The authors thus obtained a more exact volumetric control which enabled them to get a better measure of the actual quantity of acid secreted. Using this technique they obtained unexpectedly high figures for the primary acidity up to approximately 350 mN for low rates of secretion, but for increasing rates of secretion the figures decreased to about 170 mN. The authors point out the possibility that special experimental circumstances in connection with the glycine instillate might yield artificial results.

With regard to acid regulation PAVLOV (1898) maintained that the acid combines with the mucus, and he found in this an explanation why it was possible in an experiment to find lower acid values in the beginning than at the end of the secretion period although the rate of secretion was the same: the mucus was washed off and it could therefore not play the same rôle in the final phases. The importance of the mucus was also upheld by other authors (for example BOLTON & GOODHART 1931, 1933).

Another line was followed by BOLDYREFF (1914, 1934), who stressed that duodenal regurgitation, particularly of pancreatic juice, was the most important factor for acid neutralization, and he claims to have shown that if the passage through the pylorus is closed, there is no change in the acidity of the instillate. Boldyreff's theory has been criticised, especially by TEORELL (1935), who showed that Boldyreff used such a large volume of instillate in the above-mentioned experiments that one could not expect any marked changes in the acid and that his findings are, therefore, quite compatible with the theory of diffusion formulated by Teorell, of which an account will be given later on. — Here we may recall also the experiments of KESAVALU & MANN (1943), who in animal experiments let the duodenal contents pass in through the fundus ventriculi ("duodeno-fundostomy" combined with gastro-jejunosomy) with subsequent passage of the duodenal contents through the stomach. The effect on the acidity was very small. They also found that the pancreatic juice, as alkali in general, stimulated an increased acid secretion. This behaviour had also been observed earlier. KATSCH (1938), maintains that abrupt changes in the later part of the acidity curve during a test meal are caused by duodenal regurgitation. To what degree duodenal regurgitation is normally found

cannot be said to have been determined, but the conditions for it are more favourable in the case of hypochylia and achylia with imperfect pylorus control. SHAY, KATZ & SCHLOSS (1932) expressed in this connection a warning against confusing cause and effect in discussing the importance of regurgitation. (In passing it may be mentioned that BRAITHWAITE (1943) has tried to make therapeutical use of regurgitation by operating ulcer patients with cholecystogastrostomy. The results were not satisfactory and in a large number of cases the anastomosis did not remain patent.)

HOLLANDER (1938) considers acid to be neutralized by a "contaminating secretion" deriving from the mucosa of the stomach and having bicarbonate as its most important constituent. As TEORELL (1933, 1939) pointed out in his criticism of this theory, this would involve the giving off of free CO_2 during neutralization, but Teorell has been unable to find any significant CO_2 production during the acidity reduction in animal experiments.

A "Verdünnungssekretion" has been assumed also by other authors. BABKIN (1944) propounded a theory to the effect that the acid is mixed with secretion from the rest of the glandular elements which are found in the gastric mucosa (main cells, mucous cells, surface epithelium) and that it is possible to obtain gastric juice of varying composition by activating the various glandular elements in different ways. Babkin refers to the observations of KALK (1932), who by giving patients histamine and atropine simultaneously was able to obtain gastric juice of small volume and high acidity; but by combining histamine and pilocarpine he obtained gastric juice of large volume and low acidity (cf p. 237). — KATSCH & KALK (1926) have found in cases of anacidity further support for their view of the occurrence of a "wässerige Sekretion"; in these cases with histamine stimulation they obtained increased secretion and greater chloride content. In their material consisting of 13 histamine refractory cases which were also neutral red refractory (without simultaneous histamine stimulation however) they found four cases which behaved in the above mentioned way. They drew the conclusion that the faculty for "wässerige Sekretion" is the most primitive, and in the case of achylia the one which disappears last (later than the neutral red excretion) and they regard these cases as a confirmation for the independent occurrence of chloride secretion, free from acid secretion. To this we may add that in their cases one cannot exclude the possibility that an acid secretion actually took place (cf p. 238).

BABKIN refers also to KATSCH & KALK's (1926) well-known case of acid corrosion of the stomach (see also p. 189) and maintains that this case went through a stage of anacidity during which the stomach produced (under histamine stimulation) a neutral secretion with low chloride content and that acid appeared only gradually. It is difficult to

interpret these facts and it is anyhow scarcely permissible to draw any general conclusions about the normal functioning of the mucosa from the secretory response of this severely and specifically altered mucosa.

The problem of hydrochloric acid reduction in the stomach through neutralization or dilution has been treated in different ways by numerous authors but a new out-look on this problem was proposed by TEORELL (1933, 1935, 1939, 1940, 1947) in his theory of re-diffusion of acid as the most important mechanism in this connection. According to this theory the primary acidity, which is constant, is reduced in varying degrees by exchange-diffusion through the mucosa. HCl is exchanged for NaCl. Several different factors affect the rate with which the diffusion takes place:

the difference between the concentration of hydrogen ions in the stomach content and in the blood or the tissue-fluids,

the thickness of the mucosa, degree of folding, vascularisation,

the volume of the stomach content (the rate of diffusion is greater with a smaller volume on account of the relatively greater diffusion surface).

TEORELL has shown the validity of his theory in a large series of experiments on cats, in which he instilled measured amounts of acid into the closed stomach. By taking samples every 15 minutes he was able to follow the decrease of hydrogen ion concentration and the increase of cation concentration (mainly Na). The changes in volume which occurred during the experiment were insignificant and TEORELL was able to conclude from this that no neutralizing or diluting process of any importance had taken place. He further showed that the gastric mucosa is permeable to other strong or weak acids as well as to some salts. With the help of glycine buffers, Teorell, as has been mentioned before, examined primary acidity and on the assumption that the stream of secretion prevented the buffer from entering the crypts of the glands he ventured the conclusion that the diffusion, at least for the main part, takes place on the surface of the mucosa and not intraglandularly.

No large scale clinical instillation experiments have been undertaken on Teorell's lines, partly perhaps because of technical difficulties, but such few experiments as have been published up till now support Teorell's view (IHRE 1938, ELLIOT, RISHOLM, & ÖBRINK 1942, KAIJSER 1943). — IHRE used amounts of about 70 and 248 ml of 150 mN acid in two histamine refractory cases and found in the first case acid reduction to about 55 mN, in the latter to about 125 mN, that is to say a considerable difference, but one has to bear in mind that the figures are from two different patients and therefore not immediately comparable. The experiment was carried out with LAGERLÖF-ÅGREN's double tube. ELLIOT, RISHOLM, & ÖBRINK used amounts of 40, 100 and 250 ml. The experiment was carried out with a single gastric tube on two normal persons and on one with hyperacidity. They found

a reduction from 175 mN acid to 20 mN (in 70 minutes) in the first two cases. The acid in the 250 ml instillate was also reduced to about the same final level. They showed that in their cases the quantity of diffusing gramme equivalents increased in proportion to the volume. This they ascribed to the fact that the surface of diffusion increased in the same proportion (X-ray photos of instillates of 40 and 250 ml demonstrated that the ventricle had contracted about the instillate). TEORELL (1947) has returned to this problem and in summing up he states: "with a *constant* diffusion surface the acidity of a pure juice is independent of time (and hence the mode of collection) but strongly dependent of the rate of secretion; with a surface *varying* in proportion to the volume, the acidity is dependent of the time (and mode of collection) but independent of the rate of secretion." In experimental instillations a varying diffusion surface of this type will have as a consequence that the rate of acidity reduction becomes independent of the volume (Elliot, Risholm & Öbrink's cases), but Teorell emphasizes that in animal experiments the constant surface is most common. KAIJSER (1943) had occasion to carry out a unique experiment on a child 5 days old with atresia oesophagi and ligatured oesophagus and pylorus and in 2 tests with 165 and 173 mN HCl respectively obtained acid reduction to 80 and 86 mN in one hour (the case was histamine refractory).

Gastric Excretion of Neutral Red.

The fact that neutral red is excreted through the gastric mucosa was demonstrated by FULD as early as 1908. Clinically, neutral red was introduced as a test for gastric function in 1923 by GLÆSSNER & WITTEGENSTEIN, who found a far-reaching parallelism between acid secretion and the presence of neutral red in gastric juice. This fact has been the object of several investigations, the main points of which are as follows.

The method consists of an intramuscular or intravenous injection of 5 ml of 1 % neutral red solution. The first way of injection was used by Glæssner & Wittgenstein and was also more recently employed by a number of other investigators (for example DAVIDSON, WILLCOX & HAAGENSEN 1925, PIERSOL, BOCKUS & BANK 1925, KATSCH & KALK 1926, WINKELSTEIN & MARCUS 1929). But intravenous injections have the advantage of avoiding the variable time required for resorption from the intramuscular depôt into the blood. The concentration in the blood rises more quickly to a more comparable value in the various cases and this is an advantage of this method of administration, which has therefore been used by several authors (PIERSOL, BOCKUS & BANK 1925, HENNING & JÜRGENS 1930, KOBAYASHI 1925, GILLMAN 1943 etc.). Investigations were carried out either on an empty stomach or in con-

nection with various test meals and also combined with histamine (GILLMAN, HENNING, WINKELSTEIN & MARCUS, OLLEROS, ODIN etc.).

Interest is particularly centred on the appearance time of the dye in the gastric juice and as a rule this time is correlated to the degree of acidity. The interval is shorter in a case of hyperchlorhydria or a normal case than in the case of hypochlorhydria. In cases of achlorhydria the dye is generally not excreted. It may, yet, actually be demonstrated in a few of these cases though no acid seems to have been secreted. The connection with acidity suggested at an early date that the dye might be excreted through the parietal cells and most authors maintain that this is the case (GLAESSNER & WITTGENSTEIN, KATSCH & KALK, HENNING, MORRISON, GILLMAN, KOLM, KOMAROV & SHAY etc.).

GLAESSNER & WITTGENSTEIN found a time interval between injection and appearance of neutral red in the gastric juice which was correlated to the type of secretion (shortest in hyperchlorhydria and longer in hypochlorhydria). HENNING and JÜRGENS assert that the rate of excretion is on the whole fairly constant in all cases in which hydrochloric acid is produced at all with the test meal. To this one may add the remark that the rate of excretion is an indication of how quickly the dye reaches such a concentration as to become visible and that *a priori* it must be regarded as dependent on the degree of activity which the mucous membrane has at that point. One may therefore expect the technique to be of great importance, especially with regard to the question of whether the dye is injected on an empty stomach or together with a test meal. The former mode of procedure is used by Glæssner & Wittgenstein while Henning uses the latter. Another difference is that Glæssner & Wittgenstein give neutral red intramuscularly while Henning injects intravenously, which may also be thought to be of some importance. HENNING (1932) has also shown that with the use of histamine dye excretion may be provoked in animal experiments where it did not otherwise occur and that consequently histamine seems to be linked intimately with this excretion.

KOBAYASHI (1925) and MARUNO (1934) have shown in animal experiments that the neutral red excretion and acid secretion is similarly influenced by various chemicals (for instance pilocarpine and atropine) and electrical stimulation (of the vagus and gangl. coeliac.) and they conclude that dye excretion takes place mainly through the parietal cells.

ÖBRINK (1948) has carried out quantitative determinations in animal experiments with continuous intravenous administration of histamine and neutral red. In so doing he proved that the concentration of neutral red lies on a higher level for low rates of acid secretion and decreases with increasing rates of secretion. The liberation of dye, therefore, corresponded to the primary acidity (according to Linde, Teorell, & Öbrink), but not to the secondary acidity.

Of special interest are the investigations of INGRAHAM & VISSCHER (1935), which are concerned with the experimental and theoretical basis for the appearance of neutral red and other dyes in the stomach and pancreatic juice. Dyes must be divided according to their movement in the electric field and the electro-positive dyes appear in the gastric, the electro-negative dyes in the pancreatic juice. Exceptions to this rule are dyes which have either partially amphoteric characteristics or those which alter their charge during the process of oxidation or reduction. From this point of view the abovementioned authors are in opposition to Henning, who curiously considers neutral red to be an acid dye. Of particular interest seems the authors' indication of how dyestuffs may be concentrated in the gastric glandular parenchyma but are not excreted there, and they consequently regard histological methods as having only limited usefulness and as having to be treated with caution. They also observed corresponding conditions in the pancreas. The experiments were carried out on dogs and 60 different dyes were tested.

KOLM, KOMAROV, & SHAY (1945) have also shown that neutral red acts as a stimulant for the secretion of gastric juice though only to a moderate degree (experiments on dogs and cats, intravenous dose of 7.5—20 mg/kg body weight). They have likewise confirmed the earlier views that neutral red is excreted by the parietal cells and not in the pylorus end of the stomach and that there are no grounds for assuming that the mucous cells or the pepsin-secreting chief cells have anything to do with dye excretion.

But there are, of course, also divergent opinions. DAWSON & IVY (1925) maintain that they were able to produce in the dog neutral red excretion in the pylorus part of the stomach and they assumed that this happened by way of the mucous cells. No histological examinations were carried out and their findings may be compared with those of MORRISON, GARDNER, & REEVES (1935) who have pointed out that in similar experiments they found islands of parietal cells in those places of the pylorus part (or cardia) where neutral red was excreted. DAIKHOVSKY & SOLOVEI (1934) maintain that neutral red is excreted in the antrum part of the stomach, but they base this view on clinical material which is difficult to evaluate, particularly with regard to the fact that the method used is not clearly described. And it is not hard to refute their argument that a case with pylorus resection and achylia and inhibited neutral red excretion would show that the excretion takes place in the pylorus part. One might equally well regard it as proved that hydrochloric acid is secreted in the pylorus part of the stomach. — In their animal experiments Daikhovsky & Solovei have constructed an hour glass stomach with a free passage between the upper and lower parts. The secretion particularly from the pylorus part is therefore mixed. The experiment carried out in this way

does not allow any definite conclusions. — PIERSOL, BOCKUS & BANK (1925) and BOCKUS (1944) hold that the whole mass of gastric glands takes part in the excretion of neutral red but that the antrum part is more active. As evidence for this opinion they cite animal experiments (rabbit, cat, pig). Here one may point out first, that the dosage employed is large (100 mg/max. weight 8 kg) and secondly that they observed the fact that the whole surface of the gastric mucosa was coloured but not that the dye was eliminated through all different parts of it, which is altogether a different matter (vide INGRAHAM & VISSCHER). From their clinical material it may be concluded that with intravenous injection there is an agreement between the rate of dye excretion and the type of acid secretion, which is not found with intramuscular injection. The authors' assert that the excretion of neutral red in one case of achylia shows that it cannot be the acid producing cells alone which excrete the dye. HIRABAYASHI treated the gastric mucosa with 0.5 % silver nitrate and found that neutral red was not excreted although acid was secreted and concluded from this that dye elimination takes place through the main cells. MORRISON repeated these experiments but could not confirm the result.

The dye excretion in achylia is of particular interest.

GLAESSNER and WITTGENSTEIN (1925) in 7 cases of anacidity (Ewald's test meal) found that neutral red (5 ml intramuscularly) did not appear in any case.

In 15 cases of anacidity LURIA & MIKRIN (1925) administered an alcohol test meal in conjunction with which neutral red (2—4 ml) was injected intramuscularly. In 5 cases dye was set free.

KATSCH & KALK (1926) administered neutral red (5 ml) intramuscularly and found that cases of so-called anacidity (which only after the administration of histamine could secrete acid) regularly excreted neutral red. Of 13 histamine refractory cases only 2 were neutral red positive.

DAVIDSON, WILLCOX & HAAGENSEN (1925) found that every one of 8 cases of secondary anaemia and anacidity (probably not tested with histamine) excreted neutral red (injected intramuscularly). On the other hand, 8 patients suffering from pernicious anaemia were all negative to neutral red. In some of these cases the authors unsuccessfully attempted to liberate the dye by instilling $n/40$ HCl in the stomach.

HENNING & JÜRGENS (1930) obtained neutral red excretion in 3 out of 29 histamine refractory cases of achylia, among them 10 with pernicious anaemia. (They administered 5 ml neutral red intravenously.) 14 cases of anacidity (acid secretion after histamine but not after test meal) were neutral red positive. — HENNING's (1934) final conclusion is that the excretory function of the parietal cell still persists after acid secretion has ceased. At a later stage the faculty for excretion finally also disappears. He is of the opinion that the neutral red test

yields more information than the histamine test. If a case is neutral red refractory, one may as a rule conclude that the gastric mucosa is atrophic. Henning also claims to have used this test to distinguish between organical and functional achylia gastrica.

WINKELSTEIN & MARCUS (1929) use neutral red together with a test meal and sometimes also with histamine and present 60 neutral red refractory cases. They sum up their results as follows: if neutral red is excreted, it proves that the mucosa is able to secrete also HCl "whether the acid itself is excreted at that particular time or not." They also maintain that small amounts of acid may be neutralized by duodenal regurgitation and that in such cases the dye is a great help. They regard neutral red test as superior to histamine test. WINKELSTEIN (1942) has in a later investigation adopted the following normal procedure: 0.5 mg histamine and 40 mg neutral red are given intramuscularly simultaneously with a test meal of 150 ml oatmeal gruel, aspiration was made every 15 minutes. If the dye but no acid appeared, he regarded it as a sign that the stomach is nevertheless able to secrete acid and that it is only a case of temporary or false achlorhydria ("inhibition, insufficient stimulation, or neutralization of the acid"). In such cases Winkelstein repeats the test with 1 mg histamine, sometimes with other types of test meal and is then often able to demonstrate acid. He points out, however, that the dye may be present without a simultaneous acid secretion and he regards the test as a more delicate indication of the cell's activity than the usual histamine test. Among 5585 cases he found 117 with true (neutral red refractory) achlorhydria.

In his publication about the extensive investigations carried out in Norrland ODIN (1934) emphasizes, how disappointed he was with the results of the neutral red test until he discovered that it had to be combined with histamine (and in his material also with a simultaneous meal of biscuits). In this way he succeeded in showing neutral red excretion in 17 (41 %) of 41 histamine negative cases, an unusually high figure.

GILLMAN (1943, 1944) regards the neutral red test as a test *per se* and does not think it needs to be correlated with acidity. He administers 5 ml of 1 % neutral red intravenously and after 30 minutes he gives histamine and states as his experience that if neutral red is not excreted within 25 minutes, no acid will be found to be present even after the administration of histamine. He particularly recommends the test for ulcer diagnosis. — Of 24 cases of histamine refractory achylia Gillman obtained neutral red excretion in 6 (directly, without the use of stimulants). That he, among the rest, found no cases which excrete dye in conjunction with the quickly following histamine injection may appear surprising. In animal experiments KOLM, KOMAROV, & SHAY (1945) have shown that it is possible to stimulate neutral red

Table 1. *Cases of achylia, tested with neutral red, according to the literature.*

Author	Histamine refractory achylia		Achylia not tested with histamine	
	number of cases	neutral red positive	number of cases	neutral red positive
GLAESSNER & WITTGENSTEIN (1925)			7	0
LURIA & MIKIN (1925)			15	5
PIERSOL, BOCKUS, & BANK (1925) ..	8	1	12	8
DAVIDSON, WILCOX, & HAAGENSEN (1925)			8	8
KATSCH & KALK (1926)	13	2	20	20
WINKELSTEIN & MARCUS (1929) ...			16	16
HENNING & JÜRGENS (1930)	29	3	14	14
ODIN (1934)	41	17		
GILLMAN (1944)	24	6		
Total	115	29	92	71
Percentage of positive cases	25		77	

excretion in the non-secreting stomach up to six hours after injection of the dye.

OLLEROS in his investigation compared patients with tropical sprue and pernicious anaemia. If neutral red was administered intramuscularly in conjunction with histamine, the former patients excreted neutral red even when there exists a histamine resistant achylia. He regards the test as a valuable one for this differential diagnosis.

Only few histological investigations have been carried out. With special fixatives HAMPERL (1925) and MORRISON, GARDNER & REEVES (1935) in experiments on dogs succeeded in making preparations in which neutral red is shown as granula in the parietal cells and only there.

In this connection it should also be mentioned that gastroscopic examination (LERNER, ASHER & ANDREWS 1942) in 30 random cases demonstrated the appearance of localized dye excretion in 16 cases, and then only in the fundus and upper parts of the corpus but not in the cardia, antrum or pylorus. The dye appeared first as scattered spots, the size of a pinhead, which spread slowly.

It appears from this short survey that neutral red is excreted by the parietal cells; that on the whole excretion goes parallel with acid secretion; that it persists longer than the acid secretion and that this persisting ability to excrete according to several authors signifies a better prognosis in the individual case of achylia. In the literature, so far as I have been able to find, no case exists where this

ability to excrete neutral red has returned except in the case of acid corrosion of the stomach described by KATSCH & KALK (1926). This fact illustrates the graver prognosis with a negative neutral red test. One may further say that this case of acid corrosion stands in a class by itself among the instances of achylia gastrica. It is interesting to note that the authors point out that dye was excreted before any acid could be demonstrated.

As a *summary* of the investigations carried out with respect to the presence of neutral red in achylia the figures are given in table 1. They show that out of 115 cases of achylia tested with histamine 25 percent were neutral red positive. Among those not tested with histamine the frequency was far higher, viz. 77 percent. This in itself allows the conclusion that — had these cases been tested with histamine — those especially in whom neutral red was excreted would also have secreted acid and accordingly been revealed as false achylia. Even if several authors have discussed the correlation between achylia and excretion of neutral red, there seems to be no systematic investigation into the problem of whether excretion of the dye can take place without secretion of acid.

Pepsin.

Interest in the clinical aspects of pepsin has never been very great. A possible reason for this fact may be the relatively difficult and tedious technique of determination. Consequently investigations have been concentrated on the secretion of hydrochloric acid, which is easy to determine and whose presence was, furthermore, regarded as a condition for the normal functioning of pepsin.

It was thought that pepsin and hydrochloric acid are formed in different cellular elements, pepsin being formed in the main cells (HEIDENHAIN 1883) and hydrochloric acid in the parietal cells (ZIMMERMANN 1925). This view found convincing support in the experiments of LINDERSTRØM-LANG and his co-workers (1934, 1935) as well as of BOWIE and VINEBERG (1935). The former authors used frozen mucous membrane from the pig's stomach. From this they stamped out small cylindrical pieces of tissue with a punch. This was frozen and cut in sections parallel to the surface of the mucosa and in these small pieces of tissue they determined microchemically among other things the quantitative relations of acid and pepsin. From histological preparations frequency curves were calculated for the various cell types, and by combining them with the chemical results from corresponding levels it was possible to map the functions of the various types of cells in a most interesting way. BOWIE & VINEBERG showed that in the dog stimulation with histamine does not produce any very marked secretion in the main cells. On the other hand secretory pictures were obtained by vagus

stimulation and the difference is shown beautifully in the histological preparations. Stimulation of the vagus produces a great increase in pepsin secretion, and clinically this is best achieved with insulin (IHRE 1938).

It has long been observed that complete absence of pepsin is a rare phenomenon and this fact has been explained by assuming that pepsin-producing main cells are less sensitive to various deleterious influences than are the acid-producing parietal cells (RIEGEL 1886, BOAS 1925 etc.). This may be the reason why pepsin determination has been only of relatively minor importance clinically. But the greater stability of the main cells may, on the other hand, also increase the importance of the observed changes in pepsin secretion, and it has also been pointed out that a low pepsin secretion is a more reliable sign of a diseased mucous membrane than low acid secretion (POLLAND & BLOOMFIELD 1930 etc.). Complete apepsia indicates serious damage (atrophic mucosa, HENNING 1934).

There are, however, facts which point in another direction. All cell elements of the corpus glands show mitosis and are regarded as having the faculty of cell-division with the exception of the main cells, which are instead supposed to develop from mucous cells (ZIMMERMANN 1925, PLENK 1932). In certain circumstances this transformation has, however, been shown to be reversible so that the main cells revert to mucous cells, for instance in connection with the experimental use of X-ray (DAWSON 1925), in gastritis, ulcer and cancer (KONJETZNY 1928). HUEPER and DE CARVAJAL-FORERO (1944) found also that repeated small doses of X-ray left their mark on the main cells of the gastric mucosa in the dog. While it is thus possible to say that in certain cases the main cells prove more sensitive than the parietal cells, it should be remembered that this concerns morphological studies. There is no example of the occurrence of acid secretion without pepsin being demonstrated at the same time.

Achylia in pernicious anaemia has long been regarded as being particularly severe and persistent, and it is to a great extent combined with a more or less pronounced atrophy of the mucosa. The references in the literature to the occurrence of pepsin in the gastric contents from such cases are contradictory.

FABER & HOLST (1928) found in 4 cases of pernicious anaemia traces of small amounts of pepsin, and FABER (1935) maintains in his final remarks on achylia that in pernicious anaemia, various grades of functional impairment are met with and that in most cases pepsin was demonstrated. POLLAND and BLOOMFIELD (1930) found pepsin in low concentration in one case (but this value lies within the limit of error for the method, as they themselves state). HELMER, FOUTS and ZERFAS (1932) examined a series of 47 cases of anaemia perniciosa and found pepsin either absent or present only in very small amounts (the values

are about $\frac{1}{2}$ and 2 percent of the normal amounts, which they define with very wide limits). MESCHÉDE (1937) demonstrated pepsin qualitatively in three cases. DAVIES (1931) found pepsin in 4 cases out of 15 with pernicious anaemia. In one of these the diagnosis was not established with certainty and in another the figures were so low that the result cannot be regarded as convincing. Of the two remaining cases, one is stated to have received "infrequent liver therapy" without a sign of recurrence of anaemia, the other was free from symptoms without treatment for eight months (which happens sometimes also in unmistakable cases of a. pernicioso). DAVIES' final view is that pepsin is absent in cases of pernicious anaemia. Out of 10 histamine refractory cases of achylia combined with secondary anaemia 5 secreted pepsin, one of them in quite insignificant amounts and another case showed a pH-shift from 8 to 5, definitely indicating masked acid secretion.

Other authors, too, regard pepsin as lacking in such cases. KATSCH (1938) supports this view and he apparently based it on the investigations of KEMPER (1930) and BECKER (1934). The latter employed a qualitative method and categorically stated his opinion that with complete absence of acid pepsin is also lacking. This he finds exemplified in cases of pernicious anaemia and certain cases of cancer. KEMPER employed Sahli's method in an extensive investigation carried out on healthy and diseased persons and maintains that anacidity is not necessarily accompanied by aepsia but that the degree of loss of pepsin secretion is a sign of the seriousness of the process. Absence of pepsin was found in 11 cases of pernicious anaemia and 5 cases of cancer of the stomach.

IHRE investigated 6 cases of pernicious anaemia and was unable to detect any pepsin even after stimulation with insulin and rinsing with hydrochloric acid (n/10), and he concluded from this that pepsin is lacking in such cases. He used 12 cases of simple histamine refractory achylia as controls. In these, however, insulin caused secretion of acid as well as pepsin in all but two cases, and even these were positive when a renewed attempt was made several months afterwards. In one of these IHRE rinsed with acid in connection with the first (acid-negative) insulin experiment and was able to demonstrate pepsin in the rinsing fluid.

Aminopolypeptidase and Intrinsic Factor.

The reason why the presence of aminopolypeptidase in gastric contents has been examined in this connection, is especially to be found in the investigations of ÅGREN, where this author assumes that aminopolypeptidase is to be regarded as the intrinsic factor. This also necessitates a brief discussion of some parts of the literature dealing with Castle's factor.

On the basis of clinical experiments, CASTLE and his collaborators (1929, 1930, 1931), put forward the well-known theory that through the action of a so-called intrinsic factor in the gastric juice on a so-called extrinsic factor in the food an anti-anaemic factor is formed. This factor, which is necessary for the formation of normal red blood cells, is re-sorbed and stored in the liver. They further maintained that in pernicious anaemia intrinsic factor is missing, and as a consequence no anti-anaemic substance (liver factor) is found in the liver of untreated patients with pernicious anaemia, in contrast to the liver from normal persons (WILKINSON & KLEIN 1934, GOLDHAMER, ISAACS, & STURGIS 1934). On the other hand, it was possible to demonstrate the anti-anaemic factor in the liver of patients suffering from pernicious anaemia, who had received adequate liver therapy (RICHTER, IVY, & KIM 1932).

The nature of the gastric (intrinsic) factor has not been defined more closely, but Castle showed at an early date (1930) that intrinsic factor resembles an enzyme in its action and that it is active at a pH of about 7. It proved to be not identical with pepsin or any other then known enzyme in the gastric juice.

Intrinsic factor has been demonstrated in the gastric as well as in the intestinal mucosa but its distribution seems to vary in different animals and in man. MEULENGRACHT (1934, 1935) found in his investigations on the pig that anti-anaemic activity is bound up with the pyloric glands and Brunner's glands in the duodenum, while Fox & CASTLE (1942) were able to show in their experiments that in man this activity seems localised to the areas containing the fundus type of gland of the stomach.

Extrinsic factor occurs together with or as a constituent of certain kinds of protein, and the classical material, used by Castle, was muscle. In later experiments also liver has been used, which is effective by itself. Its effectiveness can be greatly increased by digestion with gastric juice (REIMANN & FRITSCH 1933), a fact which has been put to therapeutic use (SJÖGREN 1941). Liver has the added advantage that we can make use of much smaller quantities of gastric juice, since 10–20 gm of activated liver is roughly equivalent to 400 gm of activated muscle.

If one examines the literature for cases of achylia in which intrinsic factor has been determined, the results are found to be divergent and not so unequivocal as might perhaps have been expected.

CASTLE, HEATH, & STRAUSS (1931) examined 3 cases of hypochromic anaemia and one case of subacute combined sclerosis of the spinal cord and achylia (but not anaemia). The achylia was diagnosed with histamine but aspiration was never continued for more than 20–30 minutes and therefore the possibility of hypochylia cannot be excluded. With beef incubated with the gastric juice from these cases it was possible to provoke a reticulocyte response of 10–18 % in untreated pernicious anaemia cases but during the time of observation (10 days) no

erythrocyte regeneration could be demonstrated. The test patients then received meat incubated with normal gastric juice and through comparison with the resulting secondary reticulocyte reaction the authors consider that in 2 of the cases of hypochromic anaemia, the gastric juice had less effect than the normal gastric juice.

BARNETT (1932) examined 2 cases of achylia (histamine refractory for 5 and 3 years respectively) without signs of pernicious anaemia. It proved impossible to obtain a sufficient amount of gastric juice, and Barnett therefore washed the stomach for 40 minutes alternatively with NaCl-solution and $n/10$ hydrochloric acid until the combined amount was 300 ml. With this he incubated meat according to Castle's method. This preparation he afterwards gave in daily doses for 10 days. — In case 1 the reticulocyte maximum obtained was 23 % (but already before starting the test the reticulocyte value was 10 %). Within two weeks no sign of regeneration was observed, whereupon liver was given with secondary maximal reticulocyte response of the same magnitude and this was followed by quickly rising blood values. This case is somewhat difficult to evaluate. Barnett's view that it is a case of isolated reticulocyte reaction is probably right but since liver was given so quickly it is not quite certain. In case 2 there was no special effect.

HARTFALL & WITTS (1933) performed 4 tests according to Castle with gastric juice from patients with histamine refractory hypochromic anaemia. In two of these (tests 1 and 2) they used the gastric juice from 4 resp. 5 different cases of hypochromic anaemia, but in the two remaining tests only from one patient in each. It appeared difficult to obtain sufficient gastric juice and therefore it was diluted with the contents of the fasting stomach and sometimes with washing fluid (salt solution). In 2 of the tests (1 and 3) practically no effects were obtained (in case 1 the patient had a reticulocyte reaction earlier after liver per os but no regeneration; in the test the maximal reticulocyte response obtained was 1.8 %; the patient was later given meat incubated with normal gastric juice and the maximal reticulocyte response was 5.6 %). Test cases 2 and 4 showed pronounced reticulocyte reactions and were judged positive. An interesting feature is, as the authors point out, that the pepsin content varies in different cases. In test 3 with "extremely weak effect" (max. reticulocyte response 2.5 %) gastric juice with a negligible amount of pepsin was used, in case 4 on the other hand (max. ret. response 13.3 %) the test juice contained a moderate amount of pepsin. From these results we may draw the conclusion that in the first experiment it was juice from a case of true achylia, while in the second one it was from a case of hypochylia (see p. 247). The difference in effect is thus understandable.

Hartfall & Wits also consider the possibility of a correlation between the occurrence of pepsin and intrinsic factor. It was not possible to

assume any direct pepsin effect since the digestion (in test 2—4) took place at a pH of 7.

REIMANN and his co-workers SALUS and WEIL have examined the occurrence of intrinsic factor, with liver as source of extrinsic factor instead of meat. This procedure, as already stated, has the advantage that it is possible to use amounts as small as 20 gm of digested liver per day and therefore smaller amounts of gastric juice are needed. For the examination of cases with achylia this is a decided improvement, as was emphasized by Reimann & Weil, in their discussion and criticism of Barnett for adding rinsing fluid in his experiments. — To this one could add the objection that if liver after digestion shows such a great increase in activity, probably indicating an abundance of extrinsic factor, this effect should also show that the gastric juice was rich in intrinsic factor. If therefore a small amount of gastric juice from an achylia patient has this faculty, there would seem to be no reasonable objection against using a suitable amount of rinsing fluid when performing the experiment with meat, provided that the liver and meat tests are in principle comparable, as Reimann & his co-workers claim. — In 5 cases of combined spinal sclerosis with achylia (histamine and neutral red refractory) but without anaemia SALUS & REIMANN (1933) were able to show positive effect (increase of reticulocytes, hæmoglobin and red blood cells). Seven cases of pernicious anaemia with combined spinal sclerosis were also examined but here Castle's ferment could not be demonstrated. There is no further report on 4 of these cases, in 2 of the remaining ones (Nos 6 and 8) the facts reported are not sufficient for a positive diagnosis to be made. Furthermore in case 8 a test patient was used with a Hb of 91 % and a red blood cell count of 3 million, so that the negative result can hardly be regarded as conclusive. REIMANN & WEIL (1933) published two cases of achylia and subacute combined degeneration of the cord which were shown to secrete intrinsic factor (reticulocyte response but poor regeneration). — REIMANN, SALUS, & WEIL (1933) examined 8 cases of pernicious anaemia with negative result (in one of the cases there was, however, a reticulocyte increase to 8.4 % but with decreasing blood values). In comparison they cite three cases of so-called aplastic anaemia (without reaction to iron or liver) of which one had achylia. With the gastric juice of this patient a reticulocyte response of 12.4 % was obtained, but the number of erythrocytes diminished. Inconsistently enough they describe this as positive with regard to Castle's factor although "nur in geringer Konzentration." But in the main it is, of course, completely analogous with the above-mentioned case of pernicious anaemia.

GOLDHAMER (1936, 1937) collected gastric juice from various pernicious anaemia patients (by continuous aspiration lasting three hours without histamine). This he kept for months in the refrigerator until

he had enough material to perform Castle's test (150 ml gastric juice and 200 gm meat). In two cases he was able to obtain reticulocyte responses of 10 and 16 % respectively after 10 days treatment without, however, any appreciable effect on the regeneration of the blood during the period of observation. His conclusion was that the specific deficiency in pernicious anaemia is quantitative and not qualitative. Goldhamer regards it as a priori not probable that intrinsic factor is completely lacking in pernicious anaemia patients, since they are always able to produce normal blood corpuscles to a certain extent.

We may sum up the experiments discussed above by saying *that* in certain cases of achylia, not combined with pernicious anaemia, attempts to demonstrate intrinsic factor either after the digestion of meat according to Castle, or of liver according to Reimann, have been negative; *that* on the other hand the tests with the gastric juice of pernicious anaemia patients have sometimes resulted in a decided increase in reticulocytes but never caused a real remission. It does not seem possible, however, to draw any certain conclusion from this as to the presence of intrinsic factor.

ÅGREN (1942, 1943, 1944) was able to show in a series of experiments that secretin, besides its usual effect on the secretion of pancreatic juice or bile, also produces secretion from the proximal part of the duodenum and the pyloric part of the stomach. In experiments on cats he found that this secretion is rich in aminopolypeptidase. There are some grounds for suspecting that this enzyme may be the intrinsic factor, but the final proof must be a clinical one.

Liver was activated according to Sjögren's method with a preparation of aminopolypeptidase purified 100 times and then administered in daily doses corresponding to about 30 gm freshly activated liver to 4 patients with pernicious anaemia. The results were positive (ÅGREN & WALDENSTRÖM 1944). In later experiments meat was substituted which had been previously digested with pepsin and hydrochloric acid (pH 2.5) and then with aminopolypeptidase (pH 7.0). The preparation thus obtained was tested on 6 patients with pernicious anaemia. All reacted positively, two of them strongly (ÅGREN & WALDENSTRÖM 1947). Finally, 2 patients were treated with aminopolypeptidase alone. One of them showed no reaction but the other made a response which was difficult to interpret, but as Waldenström suggested, it may possibly have been due to a coincidental spontaneous remission. This question is not definitely settled however.

These clinical experiences may be regarded as strongly supporting Ågren's ideas that aminopolypeptidase is the intrinsic factor. The prospects of making a contribution to the diagnosis of pernicious anaemia by examining in a clinical material the occurrence of aminopolypeptidase in gastric juice after injection of secretin therefore seemed promising. A preliminary report on such an examination was published

by HALLÉN (1945), whose results were confirmed by ANDERSEN & FABER (1946). These Danish authors examined seven cases of pernicious anaemia and seven other cases with histamine refractory achylia. They determined aminopolypeptidase after stimulation with histamine, and in five cases after injection with secretin. In both groups they found varying values and were unable to obtain any indication that aminopolypeptidase is intrinsic factor.

CHAPTER II.

Methods.

Aspiration technique.

Intubation of the stomach is now such a commonplace operation that it needs no special comment. Yet even so simple a procedure demands a certain care and critical attitude since otherwise it may give misleading results, leading to an erroneous diagnosis.

Among those who in recent years have emphasized the importance of the control of the technical conditions with regard to the test meal PALMER, KIRSNER, & NUTTER (1940) and IHRE (1947) are especially noted. They maintain that the most important sources of error are incorrect position of the tube, pronounced regurgitation, together with copious swallowing of saliva.

As far as the tube is concerned it should be sufficiently firm and of a certain rigidity and it should consequently not be rolled up when not in use. Otherwise it easily retains a curved shape especially when it gets older, and tends to roll up in the stomach when used. The tube then does not reach the bottom of the stomach, which it is important that it should do. In different patients the position of the lowest point of the stomach varies considerably. If the tube is not pushed sufficiently far down, the result may easily be that no gastric juice is obtained at all, or only an insignificant amount, and there is a serious risk of erroneously diagnosing an achylia gastrica. During the years covered by this investigation correct intubation has shown that several cases of "histamine refractory achylia gastrica" really produced hydrochloric acid already in the fasting stomach.

To obtain gastric juice free from duodenal contents is often difficult with a simple gastric tube. For that reason double lumen tubes have been produced.

EINHORN (1918) has constructed a double tube which allows simultaneous aspiration from the duodenum and the stomach ("gastroduodenal Simultanaspirator"). The object of this was to save the patient se-



Fig. 1. Double tube (of Abbott-Rawson) with balloon and separate gastric tube.
1. duodenal intake. 2. for inflation of the balloon. 3. gastric tube.

veral intubations if an examination of the secretion both from the stomach and from the duodenum was needed. This, however, is not often the case and the instrument does not seem to have been used to any great extent.

In order to separate the gastric secretion from the pancreatico-duodenal secretion and to obtain the latter pure for analysis, LAGERLÖF & ÅGREN (1936) constructed a double tube which has been employed successfully also in gastric analysis (WELIN & FRISK, 1936, IHRE, 1938). With this tube, correctly applied, it is possible to avoid pylorus passage through continuous suction drainage from both stomach and duodenum. For obvious reason the procedure is not suitable in an instillation test, where liquid should be allowed to remain in the stomach for a certain time. Ihre also found that many such tests were spoilt by defective pylorus control.

For the experiments reported in this paper a special tube based on the double tube by ABBOTT-RAWSON¹ was therefore constructed (fig. 1) and a balloon (about 10 cm in length) attached to its smaller lumen about 20 cm from the end where the larger lumen ended. The part of the tube situated distally from the balloon lies in the duodenum. Apart

¹ There are other similar tubes which could be used. My use of this particular variant was due simply and solely to the fact that it was the only one obtainable.

from this it is also added a special gastric tube which is fixed to the double tube immediately proximal to the balloon (and only there). This carries numerous very wide perforations through which it is easily possible to introduce liquid into the stomach and also to draw it off. Especially in the emptying of the stomach it seems to be of particular advantage to have a movable tube which may, if needed, be pushed a little up or down without having to move the duodenal tube out of its position. During the war the double tube of Abbott-Rawson was very difficult to obtain and it was therefore necessary to use it as long as possible, even if it had lost its elasticity and had become difficult to manoeuvre. As an emergency measure a very fine hardened wire (piano wire) was applied along the greater part of the tube's lumen for inflation (up to the balloon) and it then remained manageable. This arrangement has obvious advantages but demands in its turn very great caution and careful supervision because it otherwise involves risks for the patient and it should under no circumstances be recommended as a routine practice. (ABBOTT (1941) has in certain cases where there were difficulties in passing the tube used a metal appliance.)

The duodenal intubation is carried out in the usual way. A throat anaesthetic was regularly given (by means of a pantocain spray) which made it very much easier to apply the tube, and the patient is considerably less troubled by this latter operation. He lies more quietly and there is a better chance of getting the tube into the correct position in a shorter time. The flow of bile is regarded as an indication that the tube has reached the right position. During the pumping up of the balloon the patient feels a twinge which may be painful and care is therefore recommended. The pressure in the balloon may go up to about 3 cm Hg (but never above 4 cm). The flow of bile through the duodenal tube should continue even after the intlation as a sign that the whole balloon lies above the papilla Vateri. As a rule this method will be sufficient without it being necessary to use X-ray for orientation. The patient is kept lying on his right side. 100 ml water are instilled and a check is kept for 15 minutes with the duodenal tube to make sure that no leakage takes place. (In earlier experiments this control was carried on for half an hour but it was soon found that if leakage takes place it was as a rule detected within 15 minutes.) Then the patient is turned on to his left side, the stomach is flushed with water two to three times, care being taken to regain the same amount. Then the instillation experiment proper can begin.

A saliva aspirator was always used and great care was taken to avoid the admixture of saliva as effectively as possible.

Similar tubes with attached balloon have been constructed by several other workers. EINHORN (1911, 1912) invented a so-called duodenal obturator in order to be able to shut off the duodenum during X-ray

examination and to get the parts of the duodenum situated above the balloon filled up with opaque contents. This seems to have had no real practical application. CASTLE, TOWNSEND, & HEATH (1930), during their investigations on the etiology of pernicious anaemia, constructed a double tube fitted with a balloon in order to be able to shut off the passage between the stomach and duodenum and obtain a pure duodenal juice.

MILLER & ABBOTT (1934) constructed a tube with an attached balloon and several lumina and have used it in some variations for examining the resorption and motility of the intestines. The balloon suitably stimulates the peristalsis. The passage of the tube is thus accelerated and it further makes possible the closing of certain parts of the gut for the study of resorption problems. It has also been used in the diagnosis and therapy of cases of ileus. — SHAY, GERSHON-COHEN, FELS, & MUNRO (1939) pioneered a tube with four lumina which was to be used in such a way that it can be fitted with two balloons, the proximal against the pylorus on the gastric side. In this way it is possible to obtain duodenal secretion free from gastric contents.

WARREN (1939) used a tube fitted with two balloons (one on each side of the pylorus). He was thus able to close the stomach. An investigation was made of the gastric resorption of glucose (WARREN, KARR, HOFFMAN, & ABBOTT 1940). — This procedure is practically identical with that used here.

Determination of Acidity.

The titration was carried out in most cases on 2 ml gastric juice (or instilled contents) up to a pH of 3.5 and 7.4 measured electrometrically. A potentiometer of the type manufactured by Radiometer with a screened glass electrode was used and an electrical mixing motor. Titration was made with 0.1-N NaOH from a burette graduated in 0.01 ml. The exactness of the method may be seen from:

	Number of cases	$M \pm \epsilon (M)$	σ	σ as % of M
Free HCl	15	126.1 ± 0.4	1.6	1.3
Total acidity	15	131.2 ± 0.5	1.9	1.4

In the case, where only a volume of 5 ml was instilled it was necessary to use a method which works with considerably smaller amounts of material. TEORELL's method (modified by Kaijser) was then made use of. One starts here with 0.1 ml gastric juice in 0.25 ml aq. dest. and titrates with 0.01-N NaOH to a similar colour with the buffer solu-

tion of pH 3.5 and 7.4 with bromophenol blue and bromo-thymol blue as indicator. The exactness of the method may be judged from:

	Number of cases	$M \pm \varepsilon (M)$	σ	σ as % of M
Free HCl	15	122.3 ± 0.2	0.9	0.7
Total acidity	15	127.9 ± 0.2	0.8	0.6

The interest is essentially centred on the exactness of the methods. For a single determination the mean error is 1.6 in respect of free acid by the electrometric and 0.9 by the colourimetric method; by the former method the error will be 1.3 percent of the mean and by the latter 0.7 percent. For total acidity the corresponding figures are 1.9 or 1.4 percent by the electrometric method and 0.8 or 0.6 percent by the colourimetric.

Determination of Chloride.

Method I. This was done electrometrically on 0.1 ml samples of gastric juice with the addition of 1 ml 1-N H_2SO_4 + 1 ml aq. dest. The electrodes were a silver wire and a glass tube with 4 % agar solution saturated with KNO_3 which serves as a connection between the test solution and a vessel containing saturated KCl into which the ordinary reference electrode dipped. An electrical mixing motor was used. Titration was carried out with 0.005-N AgNO_3 from a burette graduated in 0.01 ml. The value of the blind test was always determined and subtracted and a control test with 0.1 ml 0.1-N HCl was made in a routine way.

The reversal potential for the system lies at 275 millivolt.

Method II. The tests carried out in 1946 followed a somewhat different method: A test solution of 0.2 ml stomach juice + 10 ml 50 % acetic acid was used. As electrodes two silver wires, one dipping into the test solution the other into the silver nitrate solution of the burette, while the tip of the burette was dipped into the test solution.

The accuracy is seen from:

	Number of cases	$M \pm \varepsilon (M)$	σ	σ as % of M
Method I	20	2.748 ± 0.003	0.015	0.6
Method II	20	1.789 ± 0.003	0.014	0.8

By method I the mean error of a single determination reaches 0.015 or 0.6 percent. By method II a mean error of 0.014 or 0.8 percent was obtained.

Determination of Neutral Red.

Neutral red was here only determined qualitatively.

Several authors have used quantitative methods clinically, for example PIERSON & co-workers and DAVIDSON and his group. It is not possible to say that anything essential was gained by this procedure. One may add the remark that the latter authors have also worked out a method for getting rid of the admixture of bile and blood pigments without taking into consideration that such an admixture may well produce additional amounts of neutral red. ÖBRINK (1948) also is of the opinion that the clinical use of neutral red may be limited to qualitative tests.

The clinical value of the neutral red test seems to be restricted to cases of achylia. In other instances no advantages over acid titration should be expected. In the case of achylia the concentration always remains low and a quantitative demonstration with a series of dilutions in thin tubes as attempted by Davidson and his coworkers remains very difficult. It might be possible to determine the amount with for instance a stephen-photometer, but it is often impossible to obtain clear solutions. The dye also has a great affinity to the mucus. This means a difficult problem in all quantitative clinical determinations.

Where the dye was not seen directly, the sample was evaporated and the dry substance shaken with a few ml of 1-n HCl. To exclude the possibility of the dye having been reduced to a colourless substance hydrogen peroxide has been added in a number of cases. This procedure in no case gave a positive result.

Determination of Pepsin.

In earlier experiments many different methods were employed. Thus FABER used METT's method in which egg albumen which has been made to coagulate in small glass tubes is digested, the amount of pepsin being regarded as proportionate to the square of the length of the dissolved amount of egg albumen. POLLAND and BLOOMFIELD used edestin as a substrate and precipitated the remainder of the protein after digestion with trichloroacetic acid and, after centrifuging, read off the amount of the precipitate in a specially graduated tube. HELMER, FOUTS, and ZERFAS determined pepsin according to KOCH & McMEEKIN with an egg albumen solution of known concentration and followed the digestion refractometrically. KEMPER adapted SAHLI's method by which in a series of dilutions of the gastric juice the particular dilution is determined in which it is still possible to detect the red dye of digested carmine-fibrine. BECKER only used a purely qualitative method similar to that of KLOPSTOCK-KOWARSKI, in which a

little cube of albumen is employed as substrate and pepsin is regarded as being absent if all the albumen remains undissolved after 6—10 hours. — It is obvious that all these methods suffer from a number of inconveniences and are influenced by factors that are difficult to control.

In pepsin digestion equivalent amounts of amino and carboxyl groups are set free, and this fact has served as starting point for methods which have been devised to determine pepsin activity. SØRENSEN and WILLSTÄTTER & WALDSCHMIDT-LEITZ determined the carboxyl groups in various ways and the latter authors proceeded by titrating with alkali in alcoholic solution by which the effect of the amino groups is eliminated. This method was used by IHRE. LINDERSTRØM-LANG determined instead the amino groups by titrating in acetone with acid in alcoholic solution and this method, modified for clinical use by BENT ANDERSEN, was employed in the following experiments, an account of which will now be given.

Reagents: Casein (Merck according to Hammarsten)

n/10 NaOH

0.6-n HCl

n/10 HCl

n/40 HCl in 75 % alcohol (for titration)

naphthyl red 0.1 g/100 ml spir. conc.

acetone pur. + naphthyl red (25 dr./100 ml)

toluol

octyl alcohol.

6 gm casein are shaken up in a 100 ml volumetric flask together with 30 ml aq. dest. to which 36 ml n/10 NaOH are afterwards added in small amounts together with 0.05 ml toluol and filled up with water almost to the mark. After shaking from time to time, the casein dissolves within a few hours. 0.05 ml octyl alcohol and water up to the mark are then added. This stock solution of casein lasts for several weeks at 0°. For the final substrate-enzyme mixture a pH of 2.1 was chosen (Andersen gives 2.15). For this reason the stock solution of casein had to be made acid. With the casein used in these experiments the necessary amount was found to be 1.45 ml 0.6 n-HCl per 10 ml casein stock solution. When the casein solution is made acid, this momentarily causes considerable precipitation which, however, disappears completely if one mixes quickly by turning the tube several times. The sample of gastric juice was also corrected to a pH of 2.1, as a rule with n/10 NaOH or HCl, and the resulting dilution was afterwards corrected in the calculations. — Into each of three small test tubes 1/2 ml stomach juice is placed by means of a pipette and the whole is heated in the thermostat together with the casein solution for 10 minutes. After this 1/2 ml casein solution is added to each test tube, shaken, and to one of the tubes (the control tube) 3 ml acetone (with naphthyl red) are immediately added. The remaining two test tubes are left to digest for one hour in the thermostat at 37° and immediately afterwards 3 ml acetone are added. Titration is carried out with n/40 HCl (with a burette graduated in 0.02 ml) to match the colour in the control tube. A high pepsin concentration has the disadvantage that a slight cloudiness appears after adding acetone. This is also mentioned by Bent Andersen as a difficulty when titrating and trying to match the colour with the control tube which always remains clear. It is, however, possible to reduce this inconvenience by adding distilled water to the acetone (10—15 %). In these experiments it is therefore worked with 85 % acetone (cf. KAIJSER 1943).

The precision of the titration may be seen from the following table showing the results of 20 titrations on two different samples of gastric juice.

	Number of cases	$M \pm \varepsilon(M)$	σ	σ as % of M
First sample	20	0.180 ± 0.003	0.012	6.5
Second sample.....	20	0.214 ± 0.005	0.021	9.6

It appears that in the first of these series the degree of uncertainty expressed as the mean error of a single determination is 0.01 or 6.5 percent of the mean value. In the second series the corresponding values are 0.02 and 9.6 percent.

B. ANDERSEN proposed as unit for pepsin 1/10 of the whole amount of enzyme which under given conditions produces a proteolysis corresponding to 0.25 ml of $n/40$ HCl, and I have followed him with the difference that the amount of acid is halved, since I worked with 1/2 ml substrate and gastric juice (instead of 1 ml of each). To convert the titration figures into units of pepsin a standard curve was constructed from a series of dilutions of normal gastric juice in the usual way (cf. IHRE, KAIJSER). For this purpose histamine juice from six normal persons was used (table 2). In this way it was found that the mean value was 174 units of pepsin per ml of stomach juice.

Table 2. *Pepsin in increasing dilutions of normal gastric histamine juice (for standard curve), expressed as 1/100 ml of $n/40$ HCl, used for titration.*

Dilution	Pepsin values in the six cases					
1/2	30	30	29	32	30	28
1/4	27	26	26	29	26	26
1/5	24	24	23	28	24	23
1/6	20	22	20	22	20	21
1/10	17	18	17	18	17	17
1/20	12	12	12	14	10	11
1/50	4	7	6	7	5	5

If one examines the occurrence of pepsin in cases of achylia one has to take into account its instability in an alkaline medium. This has long been known (LANGLEY 1880—2, LANGLEY & EDKINS 1886) and NORTHROP (1932) has shown that the inactivation of pepsin occurs parallel with a denaturation of protein. IHRE also found that a sample of gastric juice with high pepsin activity lost 32 % of its proteolytic power when pH is kept at 6.5 for 15 minutes, while with a pH of 7.4 and 8.5 over the same period all activity ceased. Most of the pepsin determinations in these experiments were made on acid instillates.

Determination of Aminopolypeptidase.

The technique of intubation was the same as mentioned before except that the patient was kept lying in a right-sided position. Secretin /Pancreotest Astra/ (80 clinical units) was given intravenously, whereupon aspiration was kept up for one hour. The secretion thus obtained was scanty and highly viscous, sometimes passing through the tube with greatest difficulty. It was divided in periods of 20 minutes. In the tables the highest value obtained has been used.

Determination of aminopolypeptidase.

Substrate: 0.2 mol. solution of alanyl-glycyl-glycine (pH 7.4).

Reagent: acetone puriss. to which naphthyl red was added (10 mg/100 ml) n/20 HCl in 90 % alcohol.

The samples of gastric juice usually had a pH of about 7, otherwise corrected with a little n/10 NaOH (litmus paper). *Micromethod* after LINDERSTRØM-LANG and HOLTER (1931, 1932). During the whole experiment a pipette of a volume of 7.8 cu.mm was used for pipetting gastric juice and substrate. For each test 6 tubes (volume about 250 cu.mm) were used, of which 3 were controls. Gastric juice was put into the bottom of each test tube, to three of the tubes substrate was added, while in the control tubes the drop of substrate was put higher up on the wall so as not to be in contact with the gastric juice at the bottom. Digestion lasted for 30 minutes in a water bath of 40°, after which the process was interrupted by adding n/20 HCl (with a pipette containing 21 cu.mm). 150 cu.mm acetone and naphthyl red were then added to all the tubes. The controls were titrated with n/20 HCl to a suitable colour. The tests were then titrated to the same colour. The difference between test and control constitutes the value obtained.

The accuracy may be assessed from the following:

Number of cases	$M \pm \varepsilon (M)$	σ	σ as % of M
20	18.31 ± 0.04	0.19	1.0

CHAPTER III.

Material.

In the present study the material comprises 160 persons, 90 men and 70 women. The age distribution appears from the following:

Age	Number of cases
11—20	5
21—30	13
31—40	17
41—50	30
51—60	32
61—70	40
≥ 71	23

With regard to the secretory response to histamine the cases can be grouped as follows:

histamine refractory achylia	96
hypochylia (free HCl < 100)	41
“normal cases” (free HCl ≥ 100)	23

As to the “normal cases”, used in Chapter V, the following may be pointed out. They are normal from the point of view of secretion, but it is difficult, of course, and partly artificial to draw a line between normal cases and cases of hypochylia. For example in Ihre's normal material the figures for free HCl after histamine vary between 93 and 143 m. eq. (for the highest individual value in a period of 20 minutes) and about 5/6 of the cases lie between 110 and 140 m. eq. The difficulty in fixing a definite lower limit may be illustrated by the fact that when Ihre in his summary for this purpose uses the value of 120 m. eq. for free HCl (the mean for 60 minutes after histamine or insulin), 7 of his 24 normal cases fall below this limit. If we fix the limit at 100 m. eq. as in this paper, there is a risk of including cases, which are not “normal” from the point of view of secretion but in reality are cases of hypochylia.

Consequently cases giving free HCl < 100 m. eq. are characterized as hypochylia. — It should be noted, however, that the term hypochylia also has been used in a more restricted sense. There it stands for cases who after a caffeine test meal did not secrete acid but showed free HCl after histamine (caffeine negative hypochylia).

Signs of pernicious anaemia were present in 55 of the cases of achylia. The diagnosis was made by standard clinical methods: hyperchromic anaemia, in most cases also megaloblastic bone marrow, achylia and typical reaction to specific therapy (cf. p. 265).

CHAPTER IV.

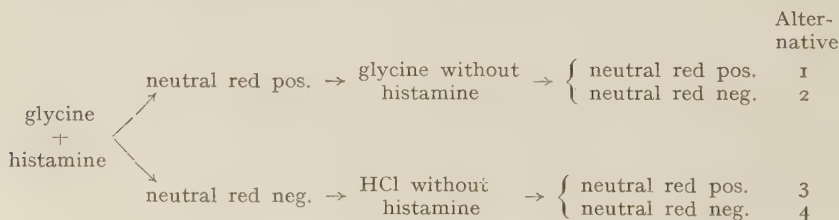
Neutral red tests.

As we know from the literature it has been demonstrated that in some forms of achylia neutral red is still excreted in the stomach. In order to check this the author has studied 43 cases of achylia (21 with pernicious anaemia and 22 other cases of histamine refractory achylia) and 18 cases of hypochylia, in which histamine provoked acid secretion but not a test meal with caffeine.

The procedure described in the chapter on methods was used. The stomach was rinsed twice with glycine solution (isotonic, 25 gm glycine "Merck" with distilled water added to 1000 ml), 50 ml of the same solution was used for each instillation experiment. At the same time was administered 5 ml of a 1 percent neutral red solution intravenously and histamine (0.1 mg per 10 kg body weight) subcutaneously.

The method of using glycine for this purpose was introduced by TEORELL (1939, 1940) and is based on the fact that this substance only to a very small extent permeates through gastric mucosa; consequently an increase in volume of an isotonic solution is a measure of the secretory volume. By dilution and buffering of the secreted acid the hydrogen ion concentration will further be decreased thereby practically preventing any reduction of the primary acidity.

The instillate is left for one hour and then aspirated quantitatively. If it contained dye, neutral red was given once more and a new glycine instillation was performed but histamine was not given. If no neutral red was brought up, 50 ml of 170 mN HCl were instilled and 5 ml neutral red given intravenously (but no histamine). The following is a schematic representation of the four possibilities which have to be taken into account in the course of this experiment.



So the following alternatives are obtained:

1. neutral red pos. in glycine without histamine
2. neutral red pos. in glycine with histamine (neg. without hist.) (table 3)
3. neutral red pos. in 170 mN HCl (neg. in glycine) (table 4 and 5)
4. neutral red neg. in 170 mN HCl

Alternative 1 in the present material is represented only by a few cases. These were all able to secrete acid even without stimulation and are, thus, of no interest in the discussion of achylia.

Table 3. *Neutral red test in glycine instillate in cases of hypochylia (histamine positive, caffeine negative).*

Case	Sex	Neutral red			
		positive after histamine		negative without histamine	
		chloride increase mN ¹	pH-shift	chloride increase mN ¹	pH-shift
1	f	73	-4.0	20	+0.7
2	f	47	-3.2	13	+0.6
3	f	42	-3.2	12	+0.2
4	f	57	-3.0	20	+0.5
5	m	51	-2.8	21	+0.5
6	m	101	-2.8	23	+0.2
7	f	41	-2.5	26	+0.6
8	m	59	-2.5	8	+1.1
9	f	69	-2.4	22	-0.2
10	f	37	-2.4	17	+0.5
11	m	22	-2.4	10	-0.3
12	f	60	-2.3	21	+0.5
13	m	46	-2.1	26	+0.7
14	m	35	-2.1	25	+0.8
15	f	23	-1.6	9	+0.9
16	m	54	-1.4	13	+0.5
17	f	50	-1.4	23	+0.5
18	m	32	-0.7	23	+0.7
Mean $\pm$ standard error of the mean		49.9 $\pm$ 4.5	-2.38 $\pm$ 0.18	18.4 $\pm$ 1.4	+0.50 $\pm$ 0.08
Standard deviation ..		$\pm$ 19.0	$\pm$ 0.78	$\pm$ 6.1	$\pm$ 0.35

¹ = millinormality.

Alternative 2 we find again in Table 3 comprising 18 cases of (caffeine negative) hypochylia. The values represent the chloride increase (expressed in milli-normality) which takes place in the glycine during the time in which the instilled fluid remains in the stomach, and the pH-

Table 4. *Cases of pernicious anaemia with neutral red test negative in glycine instillate after histamine, positive in acid instillate.*

Case	Sex	In glycine instillate	
		chloride increase mN ¹	pH-shift
19	f	22	+0.2
20	m	24	+0.2
21	f	19	+0.2
22	m	24	+0.4
23	f	9	+0.4
24	f	26	+0.5
25	m	24	+0.5
26	m	20	+0.5
27	f	28	+0.6
28	f	24	+0.6
29	f	24	+0.6
30	m	34	+0.7
31	m	24	+0.7
32	f	24	+0.7
33	m	23	+0.8
34	f	20	+0.9
35	f	18	+1.2
36	m	21	+1.3
37	m	24	+1.3
38	f	23	+1.5
39	f	20	+1.5
Mean $\pm$ standard error of the mean.....		22.6 $\pm$ 1.0	+0.70 $\pm$ 0.09
Standard deviation ...		$\pm$ 4.6	$\pm$ 0.40

¹ = millinormality

shift that takes place at the same time. The table refers to the behaviour in the cases in which histamine was given and in which neutral red was excreted. The table also refers to cases without histamine administration and in which neutral red is not excreted. From the table it will be seen that in the former cases a more or less marked degree of pH-shift takes place to the acid side, in the other cases towards the alkaline side (the mean value is -2.4 ± 0.2 respectively $+0.5 \pm 0.1$, the difference = 2.9 ± 0.2 , thus significant). (In cases 9 and 11, however, a very slight decrease in the pH occurred, which shall be discussed later, p. 211.) In the former group of cases also the increase in chloride is on a much higher level (the mean value is 49.9 ± 4.5 resp. 18.4 ± 1.4 ; the difference, 31.5 ± 4.7 , is statistically significant). The only possible conclusion seems to be that histamine has really provoked a slight

Table 5. Cases of histamine refractory achylia not associated with pernicious anaemia. Neutral red test negative in glycine instillate after histamine, positive in acid instillate.

Case	Sex	In glycine instillate	
		chloride increase mN ¹	pH-shift
40	m	47	-1.5 ²
41	f	27	+0.2
42	m	27	+0.2
43	m	30	+0.2
44	f	22	+0.2
45	m	19	+0.2
46	f	16	+0.2
47	f	25	+0.3
48	m	30	+0.4
49	m	27	+0.5
50	m	23	+0.5
51	f	22	+0.5
52	m	30	+0.6
53	f	26	+0.6
54	m	28	+0.7
55	m	28	+0.7
56	m	20	+0.8
57	f	16	+0.8
58	f	26	+0.9
59	f	23	+0.9
60	m	33	+1.0
61	m	16	+1.0
Mean $\pm$ standard error of the mean		25.5 $\pm$ 1.5	0.49 $\pm$ 0.11
Standard deviation . . .		$\pm$ 6.8	$\pm$ 0.54

¹ millinormality

² cancer with pyloric stenosis, lactic acid

acid secretion and that neutral red is excreted simultaneously. Without the help of histamine, on the other hand, no acid could be secreted and no dye excretion took place. It is thus possible to establish the close connection between the presence of hydrochloric acid and the dye. The clinical importance of this will be discussed later.

Alternative 3 is realized in table 4 and 5. In table 4 are found 21 cases of pernicious anaemia. The figures indicate an increase in chloride and a pH-shift in the glycine instillate. In contrast to the former group we get here no dye excretion after stimulation with histamine and there is also a pH-shift towards the alkaline (the average pH-shift is $+0.7 \pm 0.1$); there is therefore no sign that acid secretion takes place and also

the chloride level (mean value = 22.6 ± 1.0) lies relatively low, at about the same level as on the right side of table 3 in alternative 2.

In table 5 cases of non-pernicious achylia are to be found which in principle behave in the same way: with histamine neutral red is not excreted into glycine and here also the pH-shift (mean value $+0.5 \pm 0.1$) towards the alkaline side is noted, with the exception of case 40, where a marked drop in the pH-value took place. This may be explained from the fact that it was a case of stenosis of the pylorus with lactic acid formation, and lactic acid was also found in the instillate. (Operation proved that the stenosis was due to cancer. The rinsing of the stomach before the instillation ought to have been carried out more energetically in this case.) Otherwise the behaviour of these cases seemed unequivocal: increase in pH-value, a low chloride value (mean value 25.5 ± 1.5), and no excretion of neutral red was found.

A comparison between tables 4 and 5 shows that neither the pH nor the chloride values exhibit any statistically significant difference (the difference between the mean pH values is 0.2 ± 0.1 and between the chloride values 2.9 ± 1.8). Consequently the cases in these two tables are in this connection in principle equal.

With the instillation of 170 mN hydrochloric acid it is, however, possible to stimulate the excretion of neutral red in such cases (in cases 34 and 39 this test was not made).

A question which immediately arises is, *how concentrated the acid used must be to cause the dye to be excreted in a given case*. In cases of pernicious anaemia, where it is known beforehand that dye excretion will not take place under histamine stimulation, two instillations of acid were given at an interval of one hour. Among the 13 cases examined, in

5 cases dye could be demonstrated with 170 mN acid but not with	150 mN
1 case dye could be demonstrated with 170 mN acid but not with	120 mN
7 cases dye could be demonstrated with 150 mN acid but not with	120 mN

Alternative 4 is represented by 6 males of which 5 had pernicious anaemia. Here no dye excretion could be found even with 170 mN acid. No tests with more concentrated acids were carried out.

Discussion of neutral red excretion.

The findings seem without exception to admit the interpretation that *acid and neutral red are linked qualitatively*. The cases of hypochylia in table 3 shows this behaviour most clearly and convincingly since here both possibilities, the positive and the negative, were realized in

the same patient. Two exceptions which have already been mentioned are cases 9 and 11, where no dye could be demonstrated. It is probable, however, that the slight shift in the pH corresponds to a very insignificant amount of acid.

Titration of two samples of isotonic glycine solution (20 ml) with 170 millinormal HCl.

pH	Acid, ml, used	
6.1	I	II
5.5	0.025	0.020
5.0	0.08	0.08
4.75	0.14	0.14
4.5	0.26	0.25
4.25	0.42	0.43
4.10	0.60	0.60
3.90	0.96	0.96
3.70	1.52	1.54
3.50	2.31	2.30

If, for example, 50 ml glycine solution of the concentration used here is titrated from a pH of 6.1 to 5.5 with 170 mN HCl only 0.06 ml is required to produce this shift. In the case of the small shift which took place in the two patients mentioned (0.2 and 0.3 respectively) there must have been present even smaller amounts, and it is of course necessary to take into account certain minimal values for the appearance of the dye. In other words there may be a minimal excretion of dye which escapes detection with these simple methods. *In conclusion, it may, however, be stated, that the neutral red test is a simple and refined test for the detection of acid secretion.*

In tables 4—5 there are no signs of acid secretion: no change in pH in the glycine instillate and low chloride level following histamine stimulation. The cases therefore have true achylia and they do not excrete neutral red either. A condition of dye excretion is therefore the presence of acid. *If acid is lacking the case is neutral red refractory* whether or not the achylia is combined with pernicious anaemia.

It seems evident from what has been said before that it is not possible as has often been done (for example by WINKELSTEIN & MARCUS) to choose between neutral red and for instance histamine. In principle one must aim at *stimulating the parietal cells as powerfully as possible*, the dye serving as a guide in measuring the effect. ODIN (1934) also found that the screening capacity of the neutral red test is much increased if combined with histamine (vide p. 187). It is possible to combine the neutral red test either with histamine or insulin, to name only the two most common stimulants of gastric secretion, or with

both combined. It was not possible here to administer both together and the author therefore chose histamine. With regard to their general effect on the secretion of acid they may be regarded as almost equal, but in hypochylia and achylia in particular insulin has sometimes proved more effective. Thus IHRE (1938) succeeded in demonstrating acid with insulin in 4 cases out of 15 histamine-refractory achylia cases; HORSTMANN (1939) found the same in two similar cases. The volume of secretion is larger after insulin and in certain cases the acid secretion only after this stimulant seems to reach the threshold value at which it is technically demonstrable. One may suppose, however, that this difference does not play such a great rôle in these experiments, where the importance of the volume of secretion is largely reduced by the use of the instillation method. I have chosen histamine for its milder secondary effects. Insulin hypoglycaemia is a far more disagreeable condition for the patient even than the far from pleasant effects of histamine.

From a purely technical point of view one must bear in mind that the intubation method does not make it possible to collect every quantity of acid however small. Whether histamine or insulin is given as a stimulant, there is a group of cases with *severe hypochylia* in which the volume of secretion is so negligible that it is impossible to handle and the group of histamine or insulin refractory achylia will comprise a number of such cases, the fewer the more expertly the tube is used. The importance of detecting as many as possible of these false achylia cases is so to speak twofold: 1) in pernicious anaemia, for which diagnosis an absolute achylia may be said to be a condition, and 2) to judge the importance of a demonstrated achylia from the point of view of prognosis. It seems likely that temporary histamine-refractory achylia which has been described by CHANG, YANG, & KEEFER 1929, HENNING 1933, MÄNNLE 1936, HALLÉN 1938, IHRE 1938, LUNDHOLM 1939, VAHLQUIST 1941 a. o. belongs to this group of false achylia. Likewise, the histamine-refractory normal cases in RUFFINS & DICK'S (1939) large collection of material are possibly to a large extent cases of hypochylia, all the more so as these authors did not use fractional collection of gastric secretion. If the requirements for a diagnosis of achylia could be made more stringent, fewer cases will be diagnosed and a greater importance may be attached to the findings.

It seems indubitable that the neutral red test fulfils this need. Earlier it was already applied as an additional test in achylia diagnosis, but it was not possible to interpret it without discussing the persisting faculty for excretion by the gastric mucosa after cessation of acid formation. Experience has, however, proved its value and neutral red refractory achylia has been regarded as more serious, as has already been pointed out in the review of the literature. The real value of the test consists first and foremost in the establishment of its qualitative

connection with acid secretion, so that the presence of dye in the gastric contents shows that acid has been secreted although in such small quantities that it cannot be directly demonstrated as such in a simple way. Thus this test constitutes an easy means of demonstrating a hypochylia which is otherwise difficult to diagnose.

Dissociation between acid secretion and neutral red excretion in tuberculosis. Since it is possible to say that the presence of neutral red shows that acid is secreted, the question arises whether the absence of dye means also the absence of acid. We refer first to the reservations made on p. 211 that in practice one naturally has to take into account certain limiting values. Below a certain minimum acid secretion any dye excretion cannot be demonstrated. But apart from this we recall the results of HENNING and his collaborators (1931) that in cases of tuberculosis acid secretion is actually found sometimes even in normal amounts without the dye being excreted. The authors regard this as a specific defect of the mucosa. In order to obtain information on this point several cases of pulmonary tuberculosis and erythema nodosum have been examined. The usual technique of examination was applied. Nine of these patients proved to be neutral red positive without histamine (among them the three cases of erythema nodosum) and are of no further interest. Of the remaining cases four were neutral red positive only after histamine and three were neutral red refractory (table 6). It is remarkable that although in these three cases a shift of the pH value takes place in the glycine instillate towards the acid side, no neutral red is demonstrable. The chloride value lies on a comparatively low level but a little higher than the corresponding value in table 3 and 5.

It is possible to explain this in different ways. Henning & co-workers discussed the possibility that the dye was retained in some way like congo-red in the case of amyloidosis, but they investigated several cases of proved amyloidosis and found ordinary conditions with regard to neutral red in the gastric juice. That there is a defective retention in the parietal cells finds no support in the histamine-positive experiments or in the fact that dye can be released with acid in refractory cases. If one assumes that the pH-shifts observed here are caused by hydrochloric acid secretion, one might be tempted to draw the conclusion that the secreted acid was not sufficiently concentrated to release the neutral red, in other words we may have sometimes a primary acidity distinctly lower than is generally considered to be the case. Finally, a third possibility is that the acid in question is not hydrochloric but some other acid, perhaps lactic. Unfortunately no investigations were carried out to this effect in these cases. The material is too small to admit of any precise conclusions, but one may say that certain findings in these cases of tuberculosis must be inter-

Table 6. *Neutral red test in glycine instillate in cases of pulmonary tuberculosis.*

Case	Sex	Age	Neutral red			
			after histamine positive		without histamine negative	
			chloride increase mN ¹	pH-shift	chloride increase mN ¹	pH-shift
MÄ	f	25	93	—2.2	41	—0.9
SS	f	25	64	—2.5	40	—1.2
EV	m	39	58	—2.7	31	—0.4
KT	m	36	71	—2.6	23	—0.1
Neutral red after histamine negative ²						
ES	f	50	28	—2.3		
MJ	f	52	18	—1.8		
IS	f	59	40	—1.7		

¹ millinormality² neutral red positive in acid instillate

preted as supporting Henning's observation of a dissociation between acid secretion and neutral red excretion.

Among other cases investigated were seven with chronic arthritis (and one with Hodgkin's disease) but these have not yielded any notable results in this respect.

The Release of Neutral Red with Acid Instillate. To the views expressed above concerning the connection between acid secretion and neutral red excretion we may add observations of the fact that in neutral red refractory cases the dye may be released by a suitable acid instillate. Earlier in the literature the suggestion is found in a paper by DAVIDSON, WILLCOX, & HAAGENSEN that the permeability of the gastric mucosa for neutral red may have something to do with the presence of acid content in the stomach, and on this assumption they instilled n/40 HCl in some patients with pernicious anaemia but without any observable effects on neutral red excretion. No similar experiments are found in the literature.

In this material 56 neutral red refractory cases are to be found in whom dye excretion could be demonstrated after acid instillation (170 or 150 mN HCl). Consequently it is possible to say that the essential condition for the appearance of the dye in the stomach must be a sufficiently acid milieu at the "gastric side" of the parietal cells.

How the dye normally is liberated from the parietal cell is not known. Obviously the acid secretory process is involved of which there are several different hypotheses (e. g. HOLLANDER 1943, BULL & GRAY 1945). For example, it might be possible that the dyestuff can pass out only in the form of a salt (chloride) or that the process has to do with the electrical charge of the cell membranes and the direction of the potential drop. Finally the possibility of cellular damage might be mentioned; the cell contents being liberated and emptied out together with the dye. HEIDENHAIN (1883) advanced the hypothesis that the parietal cell should have this type of secretion (holocrine) but there is no real evidence in support of the theory.

Also the fact that it is possible in neutral red refractory cases to release dye by instilling acid can be interpreted in various ways:

- 1) the presence of acid stimulates the parietal cell to secretion;
- 2) the parietal cell is quite passive to the acid, which either enters the cell or otherwise makes it possible for the dye to pass out through the cell membrane.

The latter alternative seems most likely.

If it is possible to say that a dye may permeate through a certain membrane, it still remains to be explained why this is so. The details with regard to neutral red are, however, still obscure but it is possible to say that there seems to be complete linkage with HCl secretion. The dye is accumulated in the parietal cells but remains there until acid secretion takes place.

That this is true even for extreme cases of hypochylia, seems to be proved by these experiments (table 3). The cases in tables 4—5 also show that even if there is no longer any formation of acid the dye may be released by the administration of acid. The connection between the dye and acid is thus further established.

The results in 13 cases of pernicious anaemia (p. 210) also show that the experiment succeeds with 150 or 170 mN acid but not with 120 mN. In other words, we have to approach the value where the primary acidity is believed to lie. This seems to permit a *conclusion also in the opposite direction, namely that when the neutral red test is found to be positive the acid secreted must have been on this level although it was afterwards reduced to a greater or lesser extent*, in some cases so much that it could no longer be demonstrated by the usual methods. In other words, one might suppose that even the very insignificant amounts of acid produced are primarily present in a concentrated form. From a physiological point of view this is not unexpected, and it may be appropriate to remember how LEO as early as 1906 explained hypochlorhydria as a condition where highly concentrated acid is produced in such small amounts that the mucus is able to exert its neutralizing and diluting powers. But we now have an experimental corroboration of this. IHRE

has in a case of chronic hypertrophic gastritis shown that in a glycine instillate acid secretion took place with the same high primary acidity as normally (this case produced with the ordinary histamine test 117 mN free HCl). The present author's cases, however, exhibited serious disturbances of secretion. With regard to the regulation of acidity, however, a question that will be discussed later, one might venture the conjecture that the instilled acid does not suffer any considerable alteration in concentration during its passage through the crypts but that this happens for the most part on the surface of the mucous membrane (cf. TEORELL).

It is difficult to interpret the *six cases which did not excrete neutral red even when acid* (up to 170 mN) *was instilled* into the stomach. According to the above-mentioned facts this could mean absence of parietal cells in that zone of the mucous membrane which was occupied by the gastric contents. It would have been of interest to test with more concentrated acid. — Four of these cases were tested for pepsin and in all of them its presence was demonstrated in an acid instillate. This fact may be another indication that neutral red is not excreted by the pepsin-producing main cells.

Attempts to calculate primary acidity. It would naturally be more satisfactory if it were possible to establish the concentration of the acid secreted by direct methods. The glycine experiment seems a priori well suited to such a purpose but a serious difficulty is encountered. With the small amounts of secretion involved the sources of error are relatively great and for this reason widely fluctuating figures are obtained. An attempt to arrive at a probable value was, however, made by the following method:

Cases 1—18 (table 3) were used for these calculations. It is then necessary to know the volume of secretion. Some indication of this is obtained from the increase in volume which takes place in the glycine instillate in histamine experiments. The sources of error here are mainly:

a) on the positive side, the addition of something other than acid such as saliva, if it cannot completely be sucked off, or perhaps oesophagus secretion, mucus etc.

b) on the negative side the difficulty of emptying the stomach completely. The experiments were devised in such a way as to exclude passage through the pylorus in either direction.

Ad a) the influence of these factors is difficult to determine exactly but some indication may be obtained from the increase in volume which takes place in the case of a glycine instillate without histamine stimulation and consequently — in these cases — without acid secretion. The increase in volume must be conditioned by these sources of error and the whole depends on whether they should be regarded as equally operative during the whole time, that is to say both during and with-

out histamine stimulation. Here we are admittedly on uncertain ground. But it is possible to say that the available evidence suggests that histamine does not increase either saliva or mucus secretion (IVY 1930, ALLEY 1935, IHRE 1938). In all cases except one the histamine experiment was carried out during the first hour, and if one had to take into account the possibility that histamine, stimulation by the presence of the tube etc. causes an increase in mucus secretion, this should be more marked during the first hour. Consequently if one calculates the volume of acid secretion by subtracting the increase in volume without secretion stimulant from the increase in volume obtained with histamine, then for the above-mentioned reasons the figure may well be too high and the primary acidity calculated on this basis too low.

Ad b) the difficulty in emptying the stomach may naturally work in both directions: the stomach may contain liquid already before the beginning of the experiment. In order to reduce this possibility the stomach before each experiment was rinsed with a measured quantity of fluid and care was taken that the same amount was regained. In none of the experiments was the loss of fluid so great that after an hour a measurably smaller amount was regained than had been instilled originally.

In the histamine experiments the pH of the glycine instillate was determined at the beginning of the experiment and again after an hour. The latter samples have been titrated back to the initial pH (on 2 ml instillate) with $n/10$ NaOH. With the help of this figure and the figure for the volume of secretion the primary acidity was calculated and given in table 7.

The mean of the 18 values given in the table is 222.6 ± 21.4 (standard deviation ± 90.9). The distribution, however, is skew: 12 of the values lie between 100 and 200, the others lying above 200 and 2 above 400.

A priori it is evident that particularly in the lowest secretion volumes an error of a few millilitres makes a great difference to the figure for primary acidity. If, for instance, in case 10 one works with a limit of error of ± 1 ml of the volume, the corresponding limits for primary acidity are 238 and 297 millinormality. These figures must, therefore, be treated with great caution. It would perhaps be safer to confine oneself to the statement that no figure lies below 100 mN, but all are above the figure at which the primary acidity is usually placed with the particular reservation that some lie "absurdly" high. It seems hard to understand, why the sources of error should make themselves felt in this particular way and one is left wondering whether these high figures do not contain some measure of truth. In other words that the primary acidity may lie higher than is usually assumed. In animal experiments LINDE, TEORELL, & ÖBRINK have shown that such may be the case.

If it is possible to say that the acid secreted in even extreme cases

Table 7. *Calculated primary acidity in cases of hypochylia.*

Case	Volume of glycine instillate (ml after one hour)		Secretion volume (ml) calculated	Titration value 0.1-n NaOH, ml/2ml instil- late (aft. hist.)	Calculated primary acidity (mN) ¹
	after histamine	without histamine			
1	70	55	15	1.20	280
2	110	100	10	0.84	462
3	125	103	22	0.68	193
4	75	60	15	1.20	300
5	58	50	8	1.15	417
6	59	50	9	0.58	190
7	59	55	4	0.23	170
8	61	50	11	0.51	141
9	65	58	7	0.41	190
10	65	57	8	0.65	264
11	70	55	15	0.78	182
12	63	53	10	0.68	214
13	65	55	10	0.50	163
14	59	53	6	0.38	187
15	55	50	5	0.35	193
16	63	54	9	0.40	140
17	60	56	4	0.21	158
18	65	60	5	0.25	163

¹ millinormality

of hypochylia is concentrated, how is the reduction of the acid effected which results in such low figures in clinical tests that acid is sometimes difficult to demonstrate? This question will be discussed again in connection with the acid instillation experiments described in Chapter V.

Sources of Error. The clinical significance of the neutral red test depends largely on whether one has to consider the possibility of certain direct sources of error as dye excretion through some other mechanism. In the literature we find it stated in several places that neutral red is excreted through the mucous membrane of the small intestine and with the bile.

That neutral red is able to pass through the intestinal mucosa has been established in animal experiments by KOBAYASHI (1925—6). But it should be noted that in these experiments enormous doses were used as compared to those in use clinically. But intrinsically it is strange that an alkaline dye should be able to pass. In order to obtain some idea of its behaviour in man under the conditions employed in these tests, six cases have been examined which are described in table 8. The usual tube was passed down below the papilla Vateri. In the first

Table 8. *Neutral red in aspirated contents from the duodenum.*

Case	Sex	Age	Fasting gastric juice	Neutral red (nr), ml Histamine (h), 0.1 mg/per 10 kg body weight	Rinsing with saline (20, 20, 50 ml) after . . . min	Diagnosis
MBM	f	20	Kongo +	5 nr+h	15, 30, 60	observation for dyspepsia
EK	f	50	» +	5 nr+h	15, 30, 60	chronic arthrit.
MU	f	33	» +	5 nr+h+5 nr	15, 30, 70	» »
SS	m	19	» +	5 nr+h+5 nr	15, 30, 70	urethrit. gonorrh.
EL	f	37	» +	10 nr+h	15, 30, 60	rheumatic fever
KJ	m	21	» +	5 nr+h+5 nr	15, 30, 70	urethrit. gonorrh.

case the position was controlled by X-ray, but otherwise the procedure was to intubate until the bile stops flowing from the duodenal tube, whereas the bile came out of the gastric tube with the patient in a left position. Five ml neutral red and histamine were given in the ordinary way and aspiration was performed through the duodenal tube. In most cases no secretion was obtained and therefore the duodenum was flushed with physiological saline after 15, 30, 60 or 70 minutes (the first two times with 20 ml and lastly with 50 ml). The washings were then evaporated and mixed with 1-n HCl to which H_2O_2 had been added, but no dye was detected. In the fifth case a single dose of 10 ml neutral red was given and in the third, fourth and sixth case a second injection of 5 ml neutral red was given after 40 minutes. Thirty minutes afterwards the duodenum was rinsed with 50 ml NaCl. In none of these samples could neutral red be demonstrated in the rinsing fluid, and from these experiments one may draw the conclusion that under the conditions employed here no neutral red is excreted in the lower part of the duodenum.

However, a part of the dye is eliminated by the liver and is also regularly found in the bile. In the dosage used here this dye admixture is never so strong as to hide the colour of the bile. If one supposes that such regurgitated bile may be a source of error in neutral red tests, the simultaneous occurrence of bile colour would be expected as an indication of its origin. Vice versa, one must affirm that the presence of bile in the stomach reduces to a certain extent the diagnostic value of neutral red in the gastric contents. In a case of achylia favourable conditions for the regurgitation through defective closure of the pylorus are present. One would therefore possibly be inclined to regard this fact as a considerable limitation of the value of the neutral red test for the diagnosis of achylia. Experience shows, however, that in practice this risk seems to be negligible. WINKELSTEIN, for example, says

that he has never seen a case of pernicious anaemia excrete neutral red, and as far as can be seen in the literature there is no case of classical pernicious anaemia showing a positive test.

Occurrence of neutral red excretion among histamine refractory cases of achylia. The following question is of considerable practical importance: is it possible from the material at hand to form an opinion about the degree by which the neutral red test increases the efficiency of the diagnosis of achylia?

Table 9. *Cases of hypochylia, tested with neutral red.*

Case	Sex	Age	Free acidity/total acidity (in fractional test meal)		Diagnosis
			after caffeine	after histamine	
Neutral red test in glycine instillate positive without histamine					Carbon monoxide poisoning anaemia achrestica achylia totalis
PEB	m	28	0/10	15/37	
SW	f	55	0/15	14/28	
GJ	f	54	0/15	12/25	
Neutral red test in glycine instillate positive after (neg. without) histamine					hypochylia ulcus ventriculi? achylia gastrica cancer ventriculi
10	f	51	0/15	8/27	
12	f	80	0/12	16/30	
14	m	69	0/8	12/30	
16	m	41	0/7	5/22	

Information on this point in the literature varies somewhat and the results are not directly comparable, since the technique differs especially with regard to the fact whether neutral red was used together with secretion stimulantia (cf. p. 187). Without such stimulation HENNING found 3 neutral red positive cases among 29 histamine refractory cases, KATSCH & KALK report the corresponding figures 2 and 13. If neutral red and histamine are given simultaneously, one would expect a higher percentage of histamine refractory and neutral red positive cases (cf. ODIN). GILLMAN's view is that if no positive neutral red test (without histamine) is obtained within 25 minutes, no acid will be obtained even with histamine.

Contrary to Gillman's view, the present material contains 4 cases of hypochylia with acid secretion after histamine where the neutral red test is positive only in combination with histamine and is negative without histamine (table 9). According to Gillman one would not be justified in expecting any acid even after using histamine. Gillman's

rule thus cannot be regarded as being generally valid. Indeed, if one starts from the interpretation of the neutral red test which is put forward in this paper, the rule would appear artificial *a priori*. Three more cases of hypochylia were neutral red positive without histamine, that is to say they conformed to Gillman's rule, but no account was taken of the 25 minutes limit.

37 random cases which were histamine refractory at routine examination (table 10) were reexamined with neutral red and histamine and it was shown that no fewer than 19 (51 %), were neutral red positive (one case actually without histamine). The high figure must be ascribed to the technique used and especially to the fact that neutral red was combined with histamine. (Cases of pernicious anaemia are not included in the material.) *One may, therefore, say without hesitation that a considerably greater certainty is achieved in the diagnosis of achylia with the neutral red test. With its help half of the histamine refractory cases in this material could be proved to be instances of pseudo-achylia.*

The only other material which showed a similar frequency of positive neutral red cases among histamine refractory achylia cases was to be found in the investigations carried out by ODIN, whose findings here are confirmed and elucidated in a striking way.

Another point of interest in table 10 is that it is obviously *not possible to draw any conclusions from the value for total acidity* after a test meal with regard to the question whether it is a case of pseudoachylia or not. One would rather believe that if the total acidity is high, this is a sign that acid is actually produced but that it is neutralized in some way.

In the Norrland investigations, ODIN also found that if total acidity with Ewald's test meal was less than 8, the chance of finding acid with histamine was small (14 %) and in particular he showed that with total acidity ≥ 9 , acid could be found in 100 % after histamine and neutral red excretion occurred in a similar way: with total acidity < 5 in 25 %, total acidity 5—8 about 60 %. In this material discrepant phenomena are found. Thus not less than 13 cases with total acidity ≥ 15 were neutral red negative. It is consequently necessary to postulate here other factors than hydrochloric acid as responsible for the appearance of total acidity. In case 39 (and also in some of the others) the discrepancy may be explained by the presence of lactic acid. But there are also other possible mechanisms. KATSCH & KALK have found that with the use of bufferfree test meal in subacidity, the graphs for free HCl and total acidity diverge more and more towards the end of the secretion. Previously the authors interpreted this as being due to the admixture of mucus and the neutralization of the acid, but they revised this opinion and now regard the difference as being due to the presence of carbonate, the so-called carbonic acid error. — To sum up one may say that the *total acidity in cases of achylia for different, partly obscure, reasons may remain strikingly high. In order to make*

Table 10. *Neutral red test in 37 cases of histamine refractory achylia.*

Case	Sex	Age	Free acidity/total acidity (fractional aspiration after histamine)	Diagnosis
Neutral red test in glycine instillate positive after histamine				
1	f	49	0/5	Acroparesthesia
2	f	60	0/8	Hypochromic anaemia
3	f	51	0/16	Achylia gastrica
5	f	50	0/8	Gastrit. chron.
6	m	41	0/30	Achylia gastr., cholelithiasis
8	m	57	0/10 ¹	Mb. Schönlein
9	f	49	0/13	Neuritis
11	m	47	0/17	Achylia gastrica
13	m	63	0/30	» »
17	f	68	0/8	Lymfatic leukæmia?
18	m	58	0/12	Cancer ventriculi
HW	f	62	0/12	Achylia gastrica
RN	f	33	0/15	» »
AL	m	51	0/21	Cancer ventriculi
SL	f	74	0/8	Achylia gastrica
EJ	f	73	0/18	Polypos. ventriculi
HT	m	25	0/12	Achylia gastrica
LA	f	39	0/12	Hypochromic anaemia
RW	m	41	0/10	Carbon monoxide poisoning ²
Neutral red test in glycine instillate negative after histamine				
40	m	75	0/26 ³	Cancer ventriculi
41	f	50	0/17	Hypochromic anaemia
43	m	44	0/18	Achylia gastrica
44	f	64	0/16	Chronic arthritis
45	m	25	0/20	Achylia gastrica
46	f	64	0/20	Cholecystit. + gastrit. chron.
48	m	62	0/33	Achylia gastrica
49	m	60	0/5	Tabes dorsalis
50	m	59	0/15	Cancer ventriculi
52	m	62	0/10	» »
53	f	62	0/10	Cholelithiasis
54	m	63	0/8	Achylia gastrica
55	m	80	0/18	» »
56	m	42	0/17	Diverticulitis coli
58	f	68	0/20	Polyneuritis
59	f	32	0/20	Achylia gastrica
60	m	36	—	» »
61	m	75	0/5	Cancer ventriculi

¹ interrupted after 20 min.² neutral red positive without histamine³ lactic acid

sure that there is no concealed acid secretion present in such cases, neutral red provides a simple and trustworthy guide.

Finally it may be mentioned briefly that in 10 cases of gastric resection for ulcer 5—10 ml neutral red were administered intravenously in connection with the operation. In 5 cases a moderate, thickly mucous neutral red dye secretion was observed in the corpus part but not in the pylorus part. An attempt also, was made to show histologically which cells in the mucosa contain neutral red and several techniques were used: Hamperl's with fixation in picric acid and frozen sections, Morrison's with fixation in Susa's mixture and embedding in paraffin, as well as fixation in formalin vapour. But the author has not succeeded in finding neutral red in any cells. The reason for this may be that the neutral red dose was too small for this purpose; in animal experiments one has a considerable higher concentration to work with.

In 5 cases of cancer of the stomach which were operated on, the same experiment was made but in no case neutral red could be observed macroscopically.

CHAPTER V.

Experiments with Acid Instillation.

From the neutral red experiments reported in Chapter IV it seems probable that even in extreme hypochylia the hydrochloric acid has maximal concentration in the very moment of secretion.

As an attempt to throw some light upon these problems a number of instillation experiments have been made with 170 mN acid of varying volumes and with different patients (with normal secretion, hypoacidity and anacidity). The starting point of this work was the investigations of Teorell.

In this connection the material was divided into cases with gastric acidity ≥ 100 millinormality after histamine — these may be considered approximately normal — and cases with lower acidity which are decidedly pathological (see p. 205). The intention with the experiments was to see if there was any difference between the various groups in the acidity reduction curve of an acid instillate. As pointed out earlier the risk exists that the "normal group" will include some cases which in fact are hypochylic. If a difference nevertheless could be found between the normal group and the hypochylia this difference would not be less certain owing to that.

After the tube had been lowered in the earlier described way and the patient turned on his left side, the stomach was rinsed several times with 100 ml 170 mN hydrochloric acid, care being taken to aspirate the same amounts. In the instillation experiments various amounts

Table 11 a. *Instillation experiments with 100 ml 170 millinormal HCl in normal cases.*

Case	Age	Time in minutes						
		0	10	20	30	40	50	60
LH	71	162	157	151	145	127	116	108
ILL	16	166	161	152	135	129	119	107
LG	45	159	155	145	138	124	110	100
GP	18	150	145	135	119	116	112	105
KK	55	163	141	132	124	118	104	92
VS	26	156	146	140	130	117	106	96
EÅ	17	159	147	132	119	109	102	94
IB	38	165	162	151	140	123	114	105
HL	39	166	159	149	145	131	119	108
SB	28	166	160	156	144	135	126	111

Table 11 b.

Time in minutes	Number of cases	$M \pm \varepsilon(M)$	σ
0	10	161.2 ± 1.7	5.3
10	10	153.3 ± 2.5	7.8
20	10	144.3 ± 2.8	8.9
30	10	133.9 ± 3.3	10.3
40	10	122.9 ± 2.5	7.9
50	10	112.8 ± 2.4	7.5
60	10	102.6 ± 2.1	6.6

(namely 100, 50, 15 and 5 ml) have been used, the stomach being emptied with given intervals (for 100 ml every 10 min., 50 ml every 15 min. and for 15 and 5 ml every 30 and 20 min.), the volume measured and a fixed amount drawn off for testing (for 100 and 50 ml 3 and 2 ml, for 15 and 5 ml 1/2 ml). The experiment lasted one hour. A typical experiment is represented in figure 6 a.

Table 11 a) gives the primary values after different intervals on *normal* cases, when 100 ml 170 mN acid was instilled. On the basis of the values obtained the means and standard deviations were later calculated (see table 11 b) and fig. 2). The mean acidity value drops by about 60 units in one hour. However, there is a considerable variation, increasing with time. On the average the dispersion reaches 7.7. The variability becomes slightly smaller if we use the differences from the initial values. We thus avoid the source of variation caused by varying initial figures. This was the method used to obtain the values in table 11 c).

Table 11 c.

Difference of mean values at ... min.	Number of cases	$M \pm \varepsilon(M)$	$\sigma \pm \varepsilon(\sigma)$
0 and 10	10	7.9 ± 1.8	5.7 ± 1.3
0 and 20	10	16.9 ± 2.1	6.8 ± 1.5
0 and 30	10	27.3 ± 2.5	7.8 ± 1.7
0 and 40	10	38.3 ± 1.8	5.8 ± 1.3
0 and 50	10	48.4 ± 2.1	6.5 ± 1.5
0 and 60	10	58.6 ± 2.2	6.8 ± 1.5

As appears from this table the acidity reduction is exactly the same as in table 11 a). The new fact found is that the dispersion does not change during the course of the experiment. This implies that after 10 minutes the dispersion is about as large as it ever will be. The standard deviation of the differences lies around 6 units. To a large extent it is, probably, due to methodological errors, but to some extent changes in the mechanism of acidity reduction are responsible.

Table 12 a. *Instillation experiments with 50 ml 170 millinormal HCl in normal cases.*

Case	Age	Time in minutes				
		0	15	30	45	60
HR	24	156	147	125	97	77
GG	22	160	148	125	99	80
OO	19	163	149	128	102	80
BV	33	164	146	124	100	77
SA	40	165	155	129	110	94
EP	31	163	147	124	110	94
HS	21	164	153	134	109	90
JM	20	166	155	135	117	106
PAL	40	157	148	129	106	90
EZ	25	167	136	124	97	87

Table 12 b.

Time in minutes	Number of cases	$M \pm \varepsilon(M)$	σ
0	10	162.5 ± 1.2	3.7
15	10	148.4 ± 1.7	5.5
30	10	127.7 ± 1.3	4.1
45	10	104.7 ± 2.1	6.7
60	10	87.5 ± 2.9	9.3

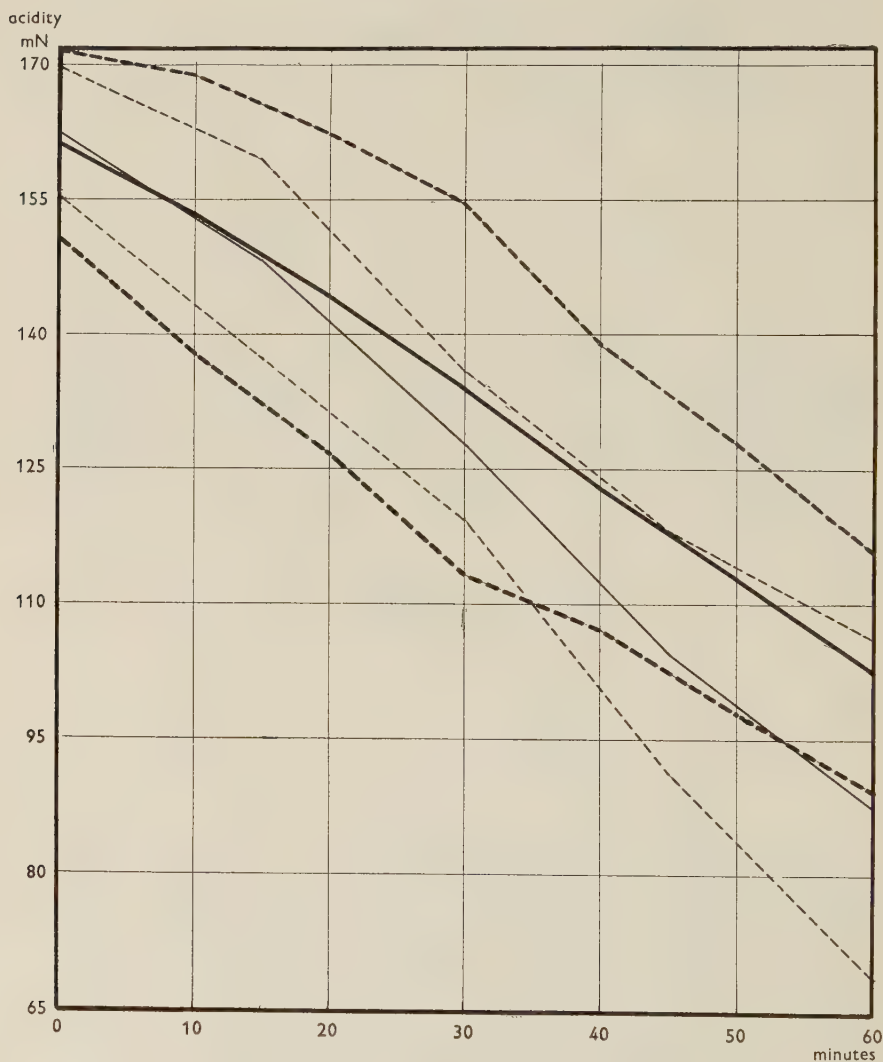


Fig. 2. Diagram of the mean values from the instillation experiments with 100 and 50 ml 170 millinormal HCl in normal cases (see table 11 b) and 12 b))

100 ml M = —————
 $M \pm 2\sigma$ = - - - - -
 50 ml M = —————
 $M \pm 2\sigma$ = - - - - -

Instillation experiments with only 50 ml 170 mN acid were also carried out in *normal* individuals (table 12 a). In these experiments the drop in acidity seems to be greater than in the previous experiments. This also appears from table 12 b) giving means, dispersions and standard deviations for the determinations. We see that in one hour the acidity drops 75 units. In these experiments the variability, as could be expected, is less than in the previous series. Fig. 2 gives a survey of the numerical values.

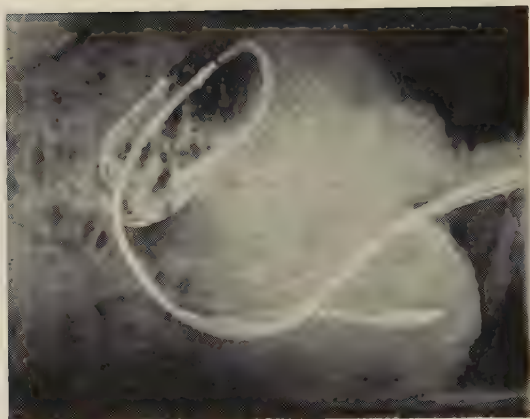
In table 13, finally, we give the figures from a few experiments carried out with 15 and 5 ml 170 millinormal HCl. This seems to indicate that the drop in acidity is still higher than in the experiments in table 12. The values are too few, however, to render possible statistical calculations.

Table 13. *Instillation experiments with 15 and 5 ml 170 millinormal HCl in normal cases.*

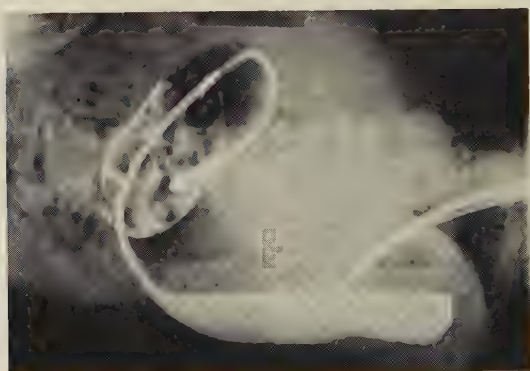
Case	Age	Time in minutes				
		0	20	30	40	60
15 ml						
IL	46	156		96		57
MJ	21	152		81		51
AL	53	145		74		34
5 ml						
IL	46	158	110		69	49
MJ	21	150	105		70	44
AL	53	144	110		64	37

This verifies TEORELL's theory of the importance of the size of an instillate. IHRE's experiments point in the same direction. ELLIOT, RISHOLM, & ÖBRINK, however, on the whole got the same curves when using different volumes (40, 100, 250 ml). On the basis of X-ray photographs of the instillate lying in the stomach, these authors suggested that the explanation of this was that the stomach contracted round the instillate in the various cases in such a way as to increase the surface of diffusion with increasing volume. To elucidate this point 5 patients with instillates of varying volume have been X-rayed and in none of them the stomach contracted but rather the fluid flowed out in a thicker or thinner layer. Figure 3 shows how various instillates (100, 50, 5 ml) have about the same longitudinal dimensions but with 100 ml 4—5 times greater thickness than with 5 ml while the 50 ml instillate occupies a medium position. Figure 4 shows the same behav-

3:1



3:2



3:3

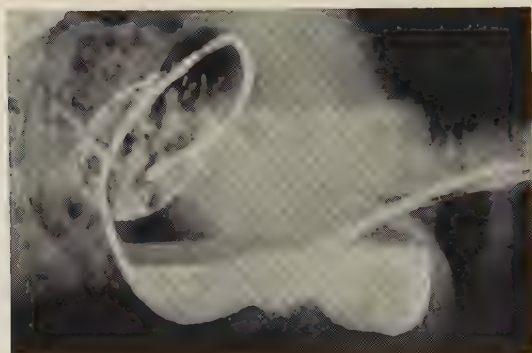
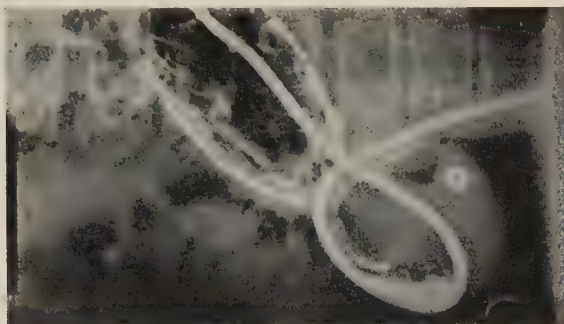
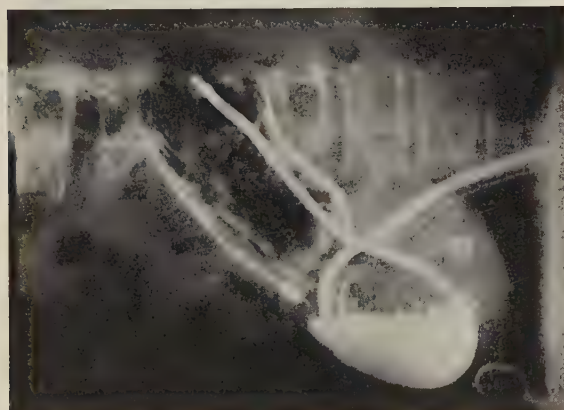


Fig. 3. X-ray picture of gastric instillate (5, 50 and 100 ml) in dorso-ventral projection, patient lying on his left side. The tip of the double tube is situated in the duodenum. The walls of the inflated balloon are partly covered by barium.

4:1



4:2



4:3

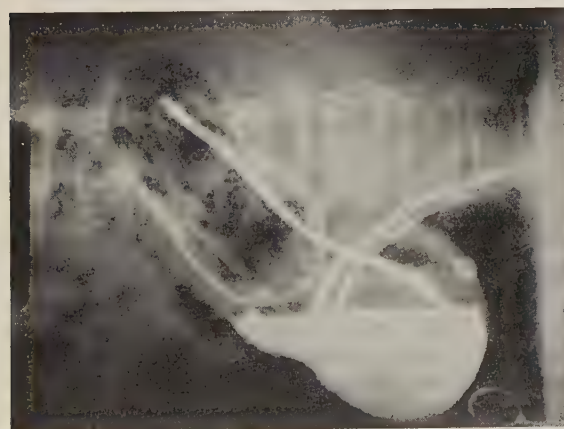


Fig. 4. X-ray picture of gastric instillate (5, 50 and 100 ml) in dorso-ventral projection, patient lying on his left side.



Fig. 5. X-ray picture of gastric instillate (50 ml) in dorso-ventral projection, patient in recumbent position. The folds of the mucous membrane are clearly marked.

ious although less clear-cut. In view of these facts it seems to be beyond doubt that the relative surface of diffusion must change considerably with a change in volume. In discussing these facts one must, however, bear in mind that the foldings of the gastric mucosa are a factor which must also be taken into account. Figure 5 may be regarded as an illustration of this. It shows a 50 ml instillate in vertical projection. The folds of the mucosa may be clearly seen through the thin contrast solution.

As has been already pointed out, ELLIOT & others found a drop in acidity which was considerably greater than in these experiments where the results are similar to the findings of IHRE and KAIJSER. The cause of this is difficult or impossible to establish, but it must be connected with the type of technique used which was not identical in any of these four investigations. One might say that the facts are naturally difficult to interpret if there is no pylorus control.

Experiments on the same person (figure 6) may serve to illustrate this point. In fig. 6 b) pylorus passage could not be prevented, the volume of the instillate rose through regurgitation (in the three last tests bile

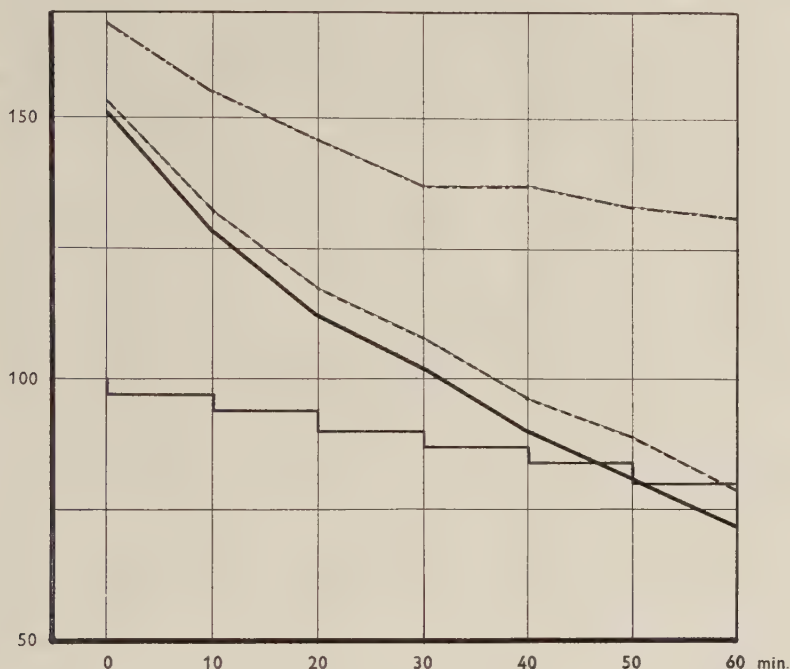


Fig. 6 a. Instillation experiment with 100 ml 170 millinormal HCl in a case of hypochlorhydria

chloride (millinormality) — · — · —
 free HCl (») —————
 total acid (») —————
 volume (ml) —————

was also found) and the curve falls much more steeply than in fig. 6 a), which show a technically successful experiment.

Figure 7 shows another case where in the latter part of the experiment there was considerable loss of volume but no sign of regurgitation and where the curve did not show any appreciable divergence from the ordinary.

Tables 14 and 15 show pathological cases.

Table 14 b) shows means, errors of the mean and dispersions for cases of what was regarded as *hypochlorhydria* according to the definition previously given. If these values are compared with values in table 11 b), containing normal cases, a difference will be observed which is statistically significant from 30 minutes and onwards (fig. 8). When compared to the normal the values for cases of hypochlorhydria lie on a lower level. If the differences from the initial values (given in

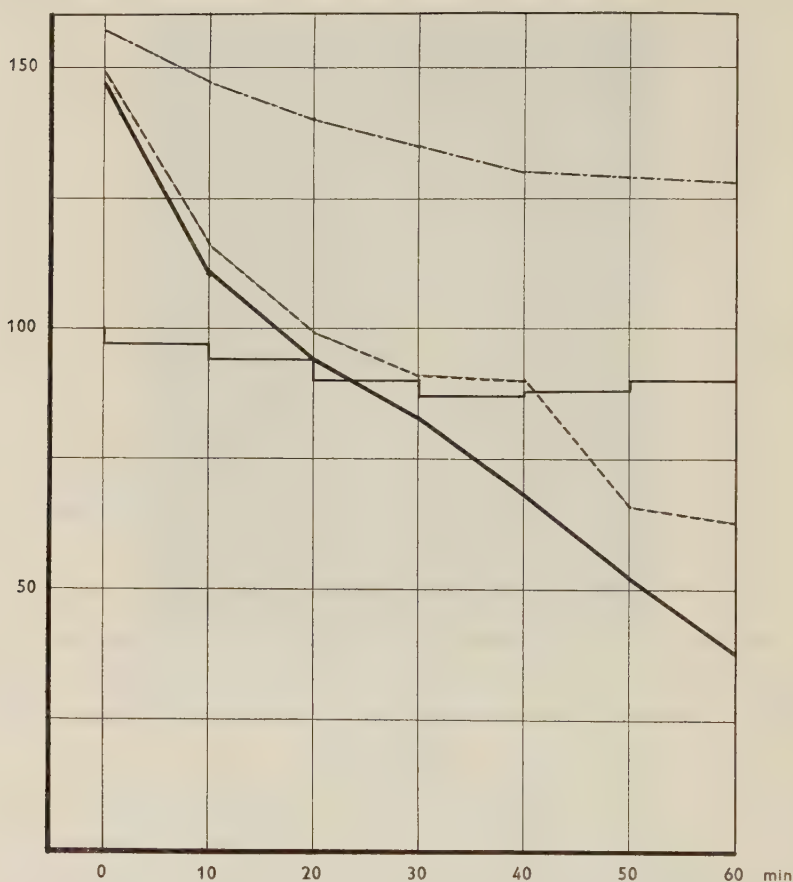


Fig. 6 b. Similar experiment as in a) but pyloric control defective (after 40 min. volume increase and bile admixture)

chloride (millinormality) — · — · —
 free HCl (») ———
 total acid (») ———
 volume (ml) ———

tables 11 c and 14 c) are compared a similar divergence will be found which also is significant from 30 min. and onwards. It is of especial interest to find out to what extent the distributions overlap. We shall use the limits $2\frac{1}{2}\sigma$ and 3σ . It then turns out that in the former case 19 percent of the values in table 14 a) lie outside the normal variability. If we use 3σ 14 percent of the values will lie outside this limit.

Repeating the same procedure with the hypochlorhydria and *achlor-*

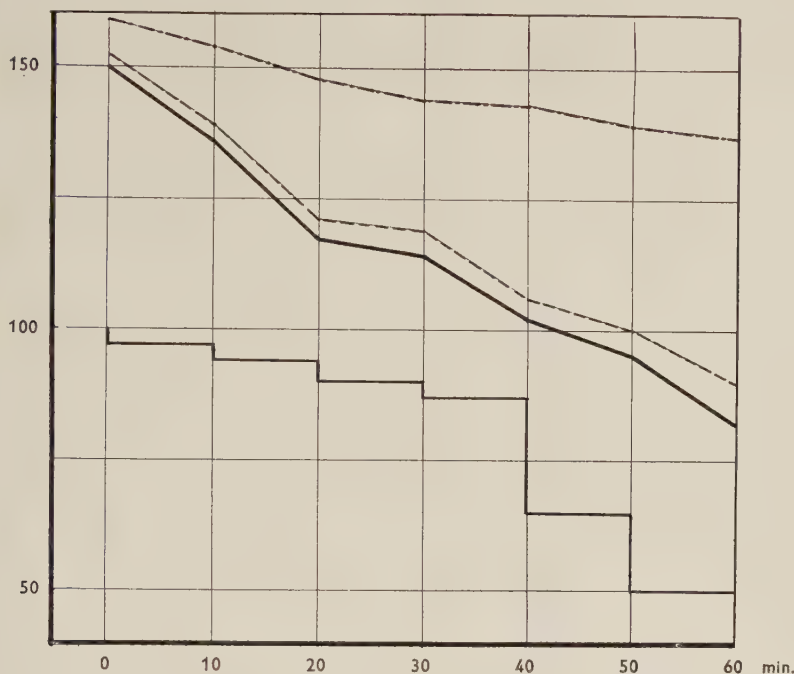


Fig. 7. Instillation experiment with 100 ml 170 millinormal HCl, technically unsuccessful (defective pyloric control, loss of volume)

chloride (millinormality) — · — · —
 free HCl (») —————
 total acid (») — — — — —
 volume (ml) ———|———

hydria values (i. e. comparing tables 14 b and 15 b), we will at no point find any significant or probable difference. The acidity reduction process seems to be slightly quicker in the achlorhydria than in the hypochlorhydria group. The number of experiments is, however, too small to prove this. Even if the differences from the initial values are compared (tables 14 c and 15 c) no significant or probable difference will be found.

The variability expressed in σ seems to be greater in the group with achlorhydria. If, however, σ in tables 14 c and 15 c are compared we find that the differences are neither significant nor probable. For that reason it is impossible to form a definite opinion on this point. Nevertheless the dispersion for achlorhydria cases consistently lies on a higher level than for the group with hypochlorhydria. If the various values were independent a statistically significant difference might

Table 14 a. *Instillation experiments with 100 ml 170 millinormal HCl in cases of hypochlorhydria.*

Case	Age	Time in minutes						
		0	10	20	30	40	50	60
SL	38	153	144	133	124	118	111	103
MA	73	160	152	136	122	106	96	87
JA	59	164	156	147	132	121	104	86
BÄ	30	157	144	124	107	97	88	82
IE	23	160	157	150	134	112	107	98
AA	51	159	146	125	117	111	101	94
GA	60	155	146	140	132	120	110	103
IH	31	156	134	119	98	94	90	81
KJ	78	151	135	124	105	95	85	77
HJ	35	164	155	139	123	115	105	90
KAL	47	157	146	137	120	96	83	75
MA	19	154	146	130	117	105	100	95
BE	24	153	132	117	108	96	89	79
EP	51	166	159	156	134	112	106	95
SN	70	165	145	122	107	105	86	82
BL	24	164	154	138	128	107	103	99
AL	50	165	154	143	124	110	102	98
JL	39	165	161	155	140	129	119	101

Table 14 b.

Time in minutes	Number of cases	$M \pm \varepsilon(M)$	σ
0	18	159.3 ± 1.2	5.0
10	18	148.1 ± 2.0	8.6
20	18	135.3 ± 2.8	12.0
30	18	120.7 ± 2.9	12.3
40	18	108.3 ± 2.4	10.1
50	18	99.2 ± 2.4	10.3
60	18	90.3 ± 2.2	9.3

Table 14 c.

Difference of mean values at ... min.	Number of cases	$M \pm \varepsilon(M)$	$\sigma \pm \varepsilon(\sigma)$
0 and 10	18	11.2 ± 1.3	5.4 ± 0.9
0 and 20	18	24.0 ± 2.3	9.8 ± 1.6
0 and 30	18	38.6 ± 2.4	10.2 ± 1.7
0 and 40	18	51.0 ± 2.1	8.9 ± 1.5
0 and 50	18	60.1 ± 2.3	9.7 ± 1.6
0 and 60	18	69.0 ± 2.2	9.3 ± 1.6

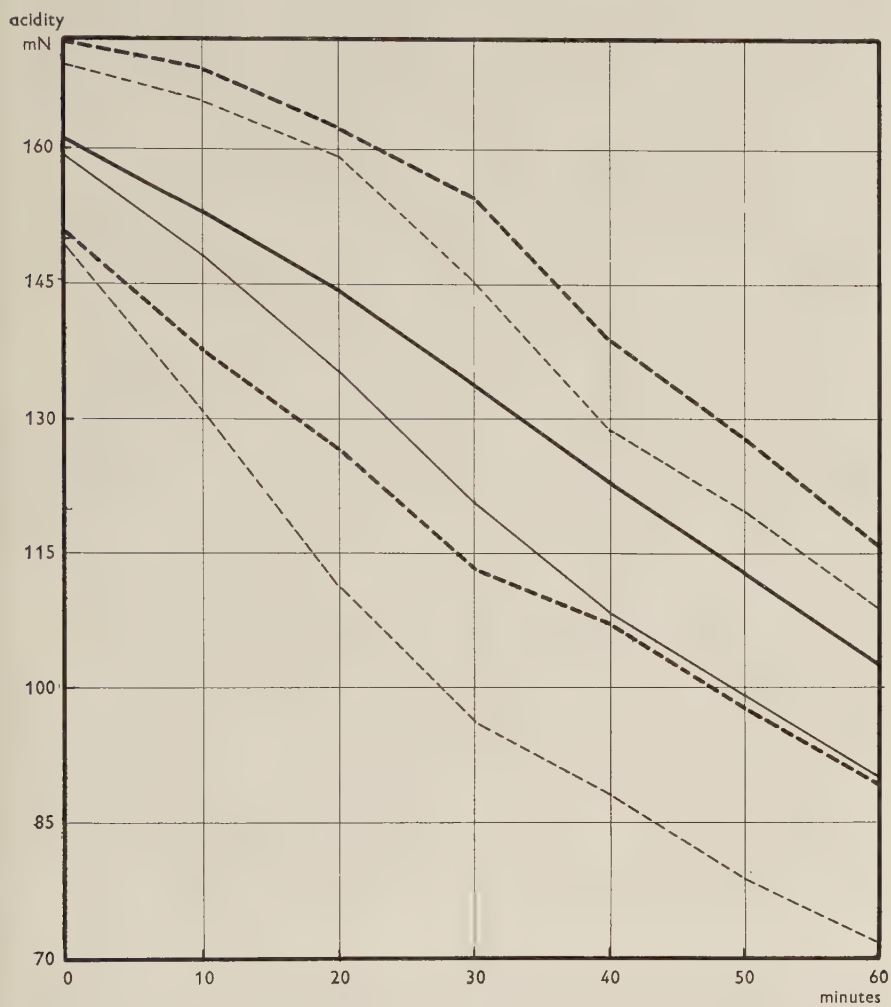


Fig. 8. Diagram of the mean values from the instillation experiments with 100 ml 170 millinormal HCl in normal cases and cases of hypochlorhydria (see table 11 b) and 14 b))

normal cases $M =$ —————
 $M \pm 2\sigma =$ - - - - -
 hypochlorhydria $M =$ —————
 $M \pm 2\sigma =$ - - - - -

Table 15 a. *Instillation experiments with 100 ml 170 millinormal HCl in cases of achlorhydria.*

Case	Age	Time in minutes						
		0	10	20	30	40	50	60
AG	65	160	150	134	125	115	102	96
SP	53	155	140	119	103	90	80	67
GL	71	163	146	125	91	74	68	60
RL	52	155	123	103	87	71	61	57
BB	47	162	146	135	123	109	93	82
LH	35	160	148	132	124	119	113	107
EK	67	165	156	149	120	105	94	91
MB	65	158	142	134	125	117	108	99
EL	56	158	141	130	123	114	105	94
DC	57	162			134			92
EL	31	159			114			95
EG	66	163			148			95

Table 15 b.

Time in minutes	Number of cases	$M \pm \varepsilon(M)$	σ
0	12	160.0 ± 0.9	3.2
10	9	143.6 ± 3.1	9.2
20	9	129.0 ± 4.3	13.0
30	12	118.1 ± 5.0	17.3
40	9	101.6 ± 6.2	18.6
50	9	91.6 ± 6.1	18.2
60	12	86.3 ± 4.7	16.2

Table 15 c.

Difference of mean values at ... min.	Number of cases	$M \pm \varepsilon(M)$	$\sigma \pm \varepsilon(\sigma)$
0 and 10	9	16.0 ± 2.2	6.7 ± 1.6
0 and 20	9	30.6 ± 3.4	10.3 ± 2.4
0 and 30	12	41.9 ± 4.6	16.1 ± 3.3
0 and 40	9	58.0 ± 6.1	18.2 ± 4.3
0 and 50	9	68.0 ± 6.0	17.9 ± 4.2
0 and 60	12	73.7 ± 4.5	15.6 ± 3.2

Difference of σ in tables 15 c and 14 c
1.3 ± 1.8
0.5 ± 2.9
5.9 ± 3.7
9.3 ± 4.6
8.2 ± 4.5
6.3 ± 3.6

be found. There is, however, a fairly strong correlation. If a value is low after 10 min. it will probably also be low after 20 min. etc. Since the strength of the correlation is unknown it cannot be corrected for when the values are added. We have therefore decided to stop at this point and refrain from carrying the calculations further.

As we have already mentioned, TEORELL also showed that the mucosa of the stomach is permeable to other acids and salts. On this basis KAIJSER carried out instillation experiments with *ammonium nitrate* and, as expected, was able to show that the ammonia concentration decreased and the chloride concentration increased. This demonstrates, therefore, diffusion in both directions through the mucosa and in order further to corroborate this fact an instillation experiment was carried out with 100 ml 1% NH_4NO_3 on a normal person, a case of hypochylia (neutral red positive), and a case of neutral red refractory achylia. All showed curves in general agreement with each other¹ (figure 9).

Teorell's theory has been criticized in some points. There is no reason for entering here into details respecting this criticism, which recently has been summarized by BABKIN (1944). The problems which are under discussion may, however, partly be illustrated by some experiments.

BABKIN considers that "the variations in the gastric acidity may be attributed to an increase or diminution in the *secretion of other groups of cells besides the parietal cells*". In support of this theory he quotes the observations of KALK (see p. 181).

In three cases a control was performed of Kalk's interesting experiment with *histamine combined with atropine and pilocarpine* respectively. His technique was so modified that both atropine and pilocarpine were administered successively in the same experiment instead of on separate occasions. Furthermore, saliva and esophagus secretion were continuously aspirated as completely as possible. A slender double lumen tube was used. One channel of the tube had been provided with perforations in the middle part which during the experiment was placed in the esophagus.

It appeared that *the great differences in volume of the gastric secretion registered by Kalk could not be verified*. This variation falls upon the saliva-esophagus secretion (fig. 10—12). Furthermore, the difference in acidity, if any, is inconsiderable. Kalk does not state whether he used a saliva sucker, but quite probably the admixture of saliva has played a large, perhaps dominating, rôle for his results. Therefore the significance of his findings is very doubtful. While stressing the importance of further experimentation, the author would like to point out that Kalk bases his own results on two atropine and two pilocarpine tests.

¹ The determination of ammonia was carried out in the Central Laboratory, the Head of which, Laborator ANDERS GRÖNWALL, I am greatly indebted to.

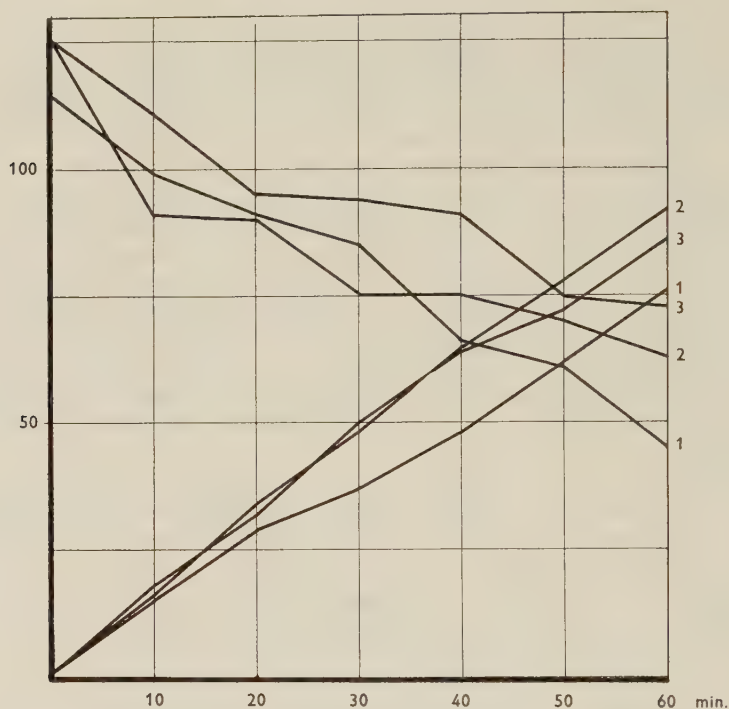


Fig. 9. Instillation experiment with 100 ml 1% NH_4NO_3 in

a normal case (1)

a case of hypochlorhydria (2)

a case of histamine refractory achlorhydria (3)

The upper curves represent ammonia, the lower chloride (both expressed in millinormality).

KATSCH & KALKS demonstration of "wässrige Sekretion" (cf. p. 181), to which Babkin also refers, can be interpreted in different ways. In their four actual cases the increase in secretion and chloride content took place 30 minutes after histamine. Thus it seems *probable that acid really was secreted*. We may compare to the cases in the upper part of table 10, which were histamine refractory at routine examination and neutral red refractory without simultaneous histamine stimulation. After histamine, however, they were neutral red positive and in reality acid was secreted (demonstrable by the use of glycine buffer).

Here, too, we may once more refer to the statement made several times by TEORELL that no changes in volume of any significance took place during his experiments, nor are any such changes registered in the authors experiments reported above.

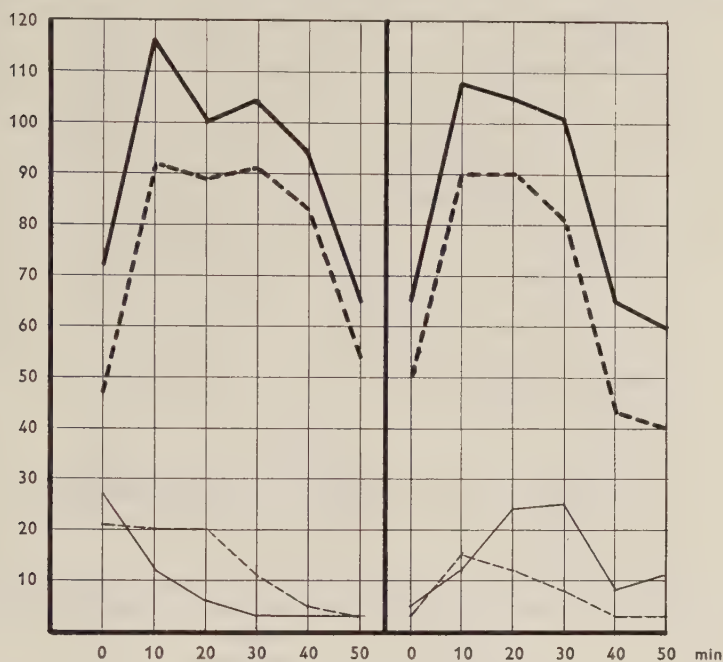


Fig. 10. Gastric secretion after subcutaneous injection of histamine 0.7 mg + atropine 1 mg (to the left) and of histamine 0.7 mg + pilocarpine 10 mg (to the right). The injections were given 10 min. before the first sample in each test.

total acid	—————
free acid	- - - - -
secretion volume (ml)	- - - - -
saliva volume (ml)	—————

About the importance of the *mucus* one may point out that here, too, the amounts are so small that for this reason alone it cannot be of any importance (TEORELL, ÖBRINK). KALK & BONIS (1932) found that the neutralizing power of the mucus was only slight and IHRE also points out that the mucus plays an unimportant part compared with the re-diffusion. GRANT (1942) carried out experiments in vitro with mucus to exclude the effect of a possible diffusion but the proportions between mucus and acid which she used (varying between 2.8 and 5.2 ml mucus to 7 ml HCl) means that the experiment is not applicable to physiological or clinical conditions. Grant considers the acidity reduction to be caused by calcium uptake from the mucus. ÖBRINK has criticized this interpretation. He pointed out that the acidity decrease observed in Grants experiments almost entirely could be explained as a matter of acidity diffusion into the mucus. In experiments on dogs

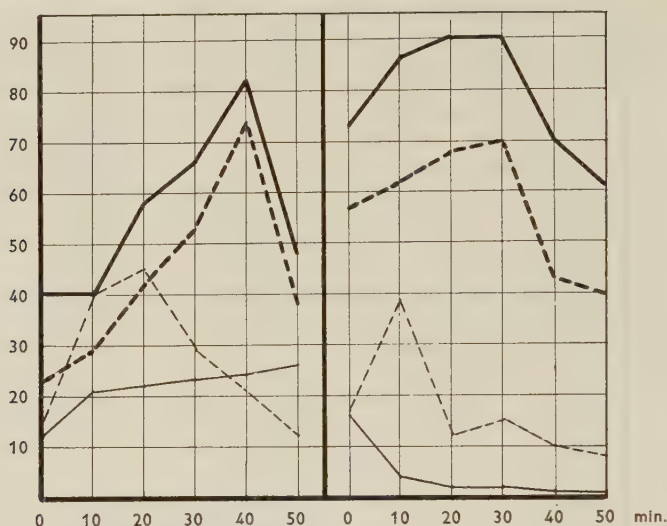


Fig. 11. Gastric secretion after subcutaneous injection of histamine 0.7 mg + pilocarpine 10 mg (to the left) and of histamine 0.7 mg + atropine 1 mg (to the right). The injections were given 10 min. before the first sample in each test.

total acid ———
 free acid - - - - -
 secretion volume (ml) — — — — —
 saliva volume (ml) —————

Öbrink also could show that the acid-binding capacity of mucus after stimulation with histamine was of very little importance.

In order to ascertain the importance of the admixture of mucus in these instillation experiments the figure for *glucosamine* was determined in five cases.¹ This does not yield any absolute figure for mucus content. The figures are, however, valuable for comparison (table 16).

Several types of secretion are represented and the cases are arranged in ascending order of glucosamine content. In the fifth case this is more than twice greater than in the second and third, but the reduction of acidity is practically the same, and we may indubitably say that *in these cases the mucus plays a negligible part* and that some other factor is decisive.

BABKIN also is of the opinion that the reduction of acidity according to TEORELL takes place only when small volumes are used and under conditions which can be realized only in exceptional cases. IHRE and ELLIOT, RISHOLM & ÖBRINK carried out experiments with volumes

¹ With kind permission of Prof. G. BLIX these tests were carried out in the Institute of Medical Chemistry by Mr E. LINDBERG, to whom I am greatly indebted. Method of ELSON & MORGAN as modified by I. NILSSON, Biochem. Zeitschr. 285: 386, 1936.

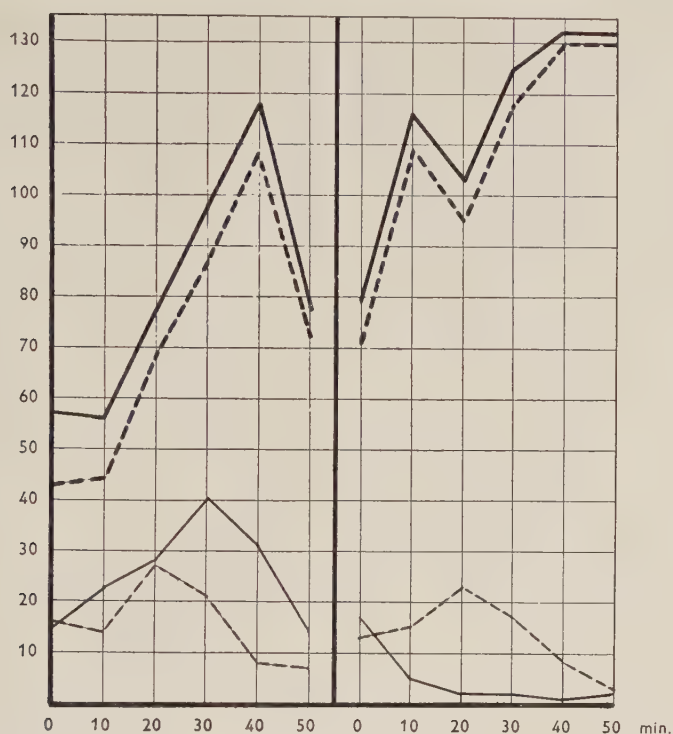


Fig. 12. Gastric secretion after subcutaneous injection of histamine 0.5 mg + pilocarpine 10 mg (to the left) and of histamine 0.5 mg + atropine 1 mg (to the right). The injections were given 10 min. before the first sample in each test.

total acid —————
 free acid - - - - -
 secretion volume (ml) ————
 saliva volume (ml) - - - - -

Table 16. *Glucosamine determination in acid instillate (100 ml 170 millinormal HCl after one hour).*

	Acidity of the instillate o/60 min.	Glucos- amine mg/ml	Free HCl/total acidity after histamine
GF normal case	160—110 (= 50)	0.066	104/120
DC pernicious anaemia	162—92 (= 70)	0.099	no free HCl
EN hypochylia	164—91 (= 73)	0.112	96/107
HL "	162—95 (= 67)	0.182	38/47
EL achylia (neutral red negative)	159—95 (= 64)	0.244	no free HCl

as large as 250 ml and yet found considerable fall in the acidity values. The volume used here (100 ml) may be said to represent an average figure for the volume of secretion during one hour after histamine stimulation and from this point of view it can hardly be called small. Under physiological conditions the acid does not lie along the great curvature, as in these instillation experiments. Here it lies in a much thicker layer than under normal conditions, in which it is secreted and spreads out over a far greater surface, so that the surface of diffusion becomes both absolutely and relatively greater. The time available for the process of diffusion on the other hand, is shorter in the latter case, how much shorter depends on the technique used (continuous outflow, taking samples at given intervals etc.). Both these factors compensate and balance each other. If one takes into account the successive reduction of acidity which is clearly seen from tables 11—15 it seems possible to explain from these experiments the more or less lowered acidity figures which are found in pathological cases and also in normal cases investigated with varying techniques. One must look for an explanation in two main directions:

1. decreased volume of secretion, illustrated in tables 11, 12, 13.
2. increase in the rate of diffusion produced in some other way illustrated in tables 11, 14, 15; one cause in this connection might be hyperaemia of the mucosa.

These experiments were devised in order to explore the two possibilities but in pathological cases we often get, of course, a combination of both and consequently a still greater change of acidity. We may recall here IHRE's hydrochloric-acid-drop experiments in which he attempted to imitate the secretion of the stomach and found that the acidity figures varied with the rhythm of the drop in the same way as he found a correlation between acidity and the rate of secretion.

CHAPTER VI.

Pepsin in cases of achylia.

The technique employed was the same as in the neutral red tests and the determinations were to a great part performed on the same cases.

Experiments described earlier (chapter IV) have shown that with regard to acid secretion and neutral red excretion achylia of the same severity as in pernicious anaemia appears also in other pathological conditions (for example sideropenia, cancer of the stomach, arthritis chron.) but also as a more isolated symptom in clinically healthy persons. It would be of interest to find out, how pepsin secretion behaves

Table 17. *Pepsin in 50 ml 170 mN¹ acid instillate from cases of histamine (and neutral red) refractory achylia, not associated with pernicious anaemia.*

Case	Pepsin units per ml instillate
40	18
43	5
44	39
45	18
46	46
49	65
50	8
52	14
53	14
55	28
57	13
59	7
60	23
GL	25
BB	2
Mean $\pm$ standard error of the mean.....	21.7 ± 4.4
Standard deviation....	± 17.1

¹ millinormal

in such cases. If it is possible to establish here a sufficiently clear distinction between cases of pernicious anaemia and other cases of severe achylia, this might conceivably help in the diagnosis of certain doubtful cases.

The pepsin values for the various groups are to be found in tables 17 and 18. They exhibit considerable dispersion but in both cases this is of the same magnitude (the difference between the dispersions, 4.8 ± 4.6 , is practically as large as its mean error). The mean values in the two series exhibit an unimportant difference (4.5 ± 6.5) which is smaller than the corresponding mean error. It can therefore be stated that with this technique *one cannot establish any difference between pernicious and other achylia and that pepsin determinations cannot be used for the diagnosis of pernicious anaemia.*

The cases in table 18 to the right were examined under somewhat different conditions. Partly the instillation of acid was combined with histamine injection, partly the instillate was left in the stomach for only one half hour (instead of one hour in other cases) (and during the preceding hour a secretin experiment had been carried out). That

Table 18. *Pepsin in 50 ml 170 mN acid (and 50 ml isotonic glycine) instillate from 21+12 cases of pernicious anaemia after one hour (to the left) and half an hour (to the right).*

Case	Pepsin units/ml acid instillate	Pepsin units/ml glycine instillate	Case	Pepsin units/ml acid instillate
19	7		ÅÅ	42
20	73	6	AA	61
21	47	2	AJ	4
22	14		VJ	41
23	39		MS	29
24	17		HJ	22
26	19		JJ	5
27	22		GP	3
28	13		HÖ	22
30	2		KD	21
31	15		ES	28
32	19	0	EV	16
33	48		Mean $\pm$ standard error of the mean 24.5 ± 5.0	
35	5		Standard deviation ± 17.3	
36	4			
37	78			
38	38	5		
GE	13			
KEK	18			
EL	49	5		
KH	11	5 (NaCl)		
Mean $\pm$ standard error of the mean 26.2 ± 4.8				
Standard deviation ± 21.9				

nevertheless practically the same pepsin values were obtained (the difference between the mean values of the two groups in table 18 is 1.7 ± 6.9) may be interpreted to indicate that there is no real pepsin secretion but merely a release of already existing amounts of enzyme, a "washing out" effect, and that this process can be completed in one half hour.

It is worth noting that nowhere in the literature are there reported so high pepsin values in pernicious achylia as in the present material. Table 19 is of interest in this connection with the pepsin values in acid instillates from 3 cases with normal acidity and 6 cases of hypochylia. As could be expected these on the whole are considerably higher. Strikingly low values do however exist. This may to some extent be due to the fact that instillates of 100 ml were used (instead of 50 ml) with a more pronounced dilution and consequently lower values for pepsin.

In 5 cases of pernicious anaemia pepsin was determined in the glycine

Table 19. *Pepsin in 100 ml 170 mN¹ acid instillate from 3 »normal cases» and 5 cases of hypochylia.*

Case		Free HCl after histamine mN ¹	Pepsin units/ml acid instillate
JF	»normal cases»	124	94
NW		124	148
DR		114	131
IE	cases of hypochylia	61	113
GA		55	40
AA		54	57
SL		63	85
JA		66	49

¹ millinormality

instillate (and in one case in a NaCl instillate). All these values are very low and on a very much lower level generally than the corresponding values in the acid instillate. This is in accordance with the above-mentioned results of IHRE. It may possibly be explained by the fact that pepsin is almost completely inactivated when the pH as in this instillate varies between 6.3 and 8.3. There are, however, factors which point in another direction. Pepsinogen, whose occurrence in the mucosa and activation by acid was conclusively proved by HERRIOT & NORTHROP (1936), is not influenced to the same extent as pepsin by an alkaline medium (LANGLEY & EDKINS 1886), and NORTHROP has shown that these changes are reversible in contrast to the changes of pepsin, where the protein becomes denatured. It must be assumed that in cases of pernicious achylia there is no possibility for pepsinogen, if present, to become activated by acid, nor is an inactivating influence of the medium to be feared. After activation (which demands a $\text{pH} \leq 4$) one could therefore expect about the same pepsin value in a glycine instillate as in an acid one. In all cases glycine has been instilled prior to acid and therefore the insignificant amounts of pepsin in the glycine cannot be regarded as due to mechanical causes (that is to say that there is a certain amount of pepsin to be found on the surface of the mucosa which is only washed off by the instillate). Nor is there any reason to believe that the glycine in itself inactivates or inhibits pepsin secretion. In cases of hypochylia the author has been able to demonstrate the presence of pepsin in fairly considerable amounts also in glycine instillates (52, 59, 61, 80, 109 units of pepsin per ml).

The titre values of the glycine samples from pernicious anaemia cases (table 18) are respectively 0.04, 0.03, 0.05, 0.05, 0.04. (Titre values and pepsin units are not fully parallel in the various cases because of the varying correction for dilution.) In these titrations the degree of

uncertainty is 0.02 ml. We are therefore allowed to conclude that these figures are not likely to prove the actual presence of pepsin.

To throw further light on this question some experiments have been made to induce pepsin secretion in a glycine instillate by means of insulin in patients with pernicious anaemia.

16 I.U. of insulin were given intravenously together with 5 ml neutral red (1 % sol.). Stomach lavage was carried out several times with isotonic glycine solution and after 30 minutes, when the activity of insulin begins to be noticeable, 50 ml glycine were instilled and left in the stomach for one hour. After rinsing with 50 ml 100 mN HCl several times another 50 ml of the acid were instilled and left for about one half hour, whereupon the whole procedure was repeated with 170 mN HCl for another half hour. An ordinary single stomach tube was used for these experiments and one could therefore not avoid admixture of duodenal contents. Macroscopically visible admixture of bile also occurred. The glycine instillate was corrected to a pH of 2.1 and stored in the refrigerator for about 24 hours before the determination of the pepsin content. The results will be found in table 20.

Table 20. *Cases of pernicious anaemia. Pepsin in glycine and acid instillates (100 and 170 mN¹).*

Case	Pepsin units/ml instillate		
	Glycine	100 mN ¹ HCl	170 mN ¹ HCl
EF	< 1	3	40
MG	0	3	3
EL	0	0	38
IP	0	0	23
JH	< 1	4	35
JG	0	2	29
EÖ	0	—	41

¹ millinormal

In none of the seven cases was pepsin secreted into the glycine instillate. In 100 mN HCl pepsin is found in such small quantities that its presence cannot be regarded as conclusively proved. In 170 mN HCl the pepsin values are manifest in all cases except one. It seems justifiable, therefore, to draw the conclusion that pepsin secretion demands the presence of acid in considerable concentration. How concentrated the acid must be does not emerge from this experiment, but it seems that 100 mN acid is not sufficient and this may be the reason, why IHRE did not find pepsin in any of his patients with anaemia perniciosa.

If we turn to the problem of where exactly the acid intervenes in the mechanism of secretion several possibilities may be discussed.

One is that the acid enters the main cells and that intracellular activation of pepsinogen to pepsin takes place which latter enzyme is then secreted. In this case it is assumed that the cells have the power of secreting pepsin but not pepsinogen. But there is no plausible reason, why this should be the case and it would seem more probable that there is some other way in which acid enables secretion to take place together with a simultaneous activation of pepsinogen. However this may be, it follows that *one cannot assume an isolated secretion of either pepsin or acid; they always occur simultaneously* even though the quantitative relationships change (as in histamine and insulin secretion). This behaviour may also explain the statements found in the literature that in achlorhydria pepsin secretion can be provoked by histamine (e. g. OSTERBERG, VANZANT and others 1936). In such cases a latent acid secretion may be assumed and in this way the histamine effect would be easily explained. From the functional point of view, therefore BECKER's conception, which has been cited above, may be regarded as correct, namely that pepsin is not found where hydrochloric acid is completely absent. Bearing in mind that pepsin secretion is thus bound up with acid secretion *it is also easy to see how well the anatomical position of the parietal cells is adapted to their function, scattered as they are here and there among the main cells.*

The pepsin experiments have further shown that lack of pepsin is no characteristic symptom of pernicious anaemia. All cases of true achylia lack pepsin in the standard test. Most of them produce pepsin if acid in the necessary concentration is administered. Some authors are inclined to regard apepsia as a help for the prognosis in a case of achylia. It must, however, be remembered that absence of pepsin in a standard sample of gastric juice probably only means that acid is not secreted. Only if pepsin is lacking in a suitable acid instillate is it possible to say that pepsin cannot be secreted (in that part of the mucosa which is reached by the instillate).

A further question is the following: How is the old observation that acid secretion disappears before pepsin secretion to be explained if the latter is only possible in the presence of rather concentrated acid? They should, of course, both disappear simultaneously. Here one may draw a parallel with neutral red excretion. The acid secretion may be so small that a pH below 3.5 is never reached in the aspirated gastric juice. Consequently it is not detected as free acid in titration but makes pepsin secretion possible all the same. DAVIES found pepsin in half of his cases of simple histamine refractory achylia, as already mentioned. In the author's material neutral red positive cases occur with the same frequency (see p. 221). *The presence of pepsin, as of neutral red, in a given case of achylia thus shows that acid has been secreted and that the achlorhydria is not absolute.* But this material, nevertheless, shows the correctness of the view that pepsin secretion persists longer, since

patients with serious achylia, with or without pernicious anaemia, generally produce pepsin in an acid instillate. From the point of view of nomenclature one might say that *the distinction between achlorhydria and achylia ceases to exist* (that is to say if it is based on an examination of ordinary gastric juice samples without the instillation of concentrated acid).

At this point some cases of special interest may be cited namely those, where pepsin continues to be demonstrable although the achylia is neutral red refractory even in 170 mN HCl (table 21).

Table 21. *Pepsin in acid instillate of varying strength. (Cases neutral red negative even in 170 mN¹ HCl).*

Case	150 mN HCl pepsin units/ml	170 mN HCl pepsin units/ml
KEK	11	18
GE	6	13
KH		11
EL		49

¹ millinormal

In 2 of the 4 cases the pepsin values are decidedly low, in one of them relatively high. No far-reaching conclusions should be drawn from this small number of observations. It might be regarded, however, as indicating that during the process of increasing atrophy the parietal cells have disappeared before the main cells.

As has been pointed out earlier, the view that acid and pepsin are secreted from separate cells finds support in these cases, where there is an undeniable dissociation between neutral red and pepsin in the acid instillate.

We might be tempted to conclude from these findings that they throw new light on, or give fresh importance to, acid therapy of achylia cases. The practitioner, wishing to have a rational basis for his therapy, must reject this conclusion however regretfully. Even if it might be possible that the administration of acid before a meal induces a certain amount of pepsin secretion it is certainly impossible to produce by any reasonable dosage of acid a sufficiently low pH in the stomach content during the meal to give the pepsin an opportunity to work. The attitude towards this therapy during recent years has therefore been that one cannot count on restoring the acid medium (IHRE 1940, FRANKEL 1945). Frankel states that for an ordinary meal 15–20 ml 1 % HCl are required to keep up acidity. In this connection an experiment was made on case 60:

With a 100 gm meal of meat varying amounts of glutamic acid were

administered and the pH in the collected gastric content was determined:

100 gm meat	+	0.60 gm glutamic acid	after	1 hr	pH 6.5
100 »	»	+ 1.80 »	»	1 »	» 5.9
100 »	»	+ 3.60 »	»	10 min.	» 4.14
				» 30 »	» 4.96
				» 60 »	» 4.53

The ordinary dosage mentioned for this preparation is 1—2, at the most three tablets of 0.3 gm at one time, while the dosage used in this experiment was 6—12 times greater. If one realizes that 0.3 gm glutamic HCl corresponds in its effect to about 35 drops acid hydrochlor. dil. the dosage employed here corresponds to 420 dr. acid (= 21 gm).

Consequently, acid therapy in order to enable pepsin digestion in cases of achylia must be regarded as impracticable unless we are prepared to adopt HENNING & NORPOTH's proposal, and introduce a very thin rubber tube through the nose into the stomach and so administer large amounts of $n/10$ HCl, or even stronger acid, during the meal. Doubtless it would be possible to perform a real substitution therapy in this way but the technique makes unreasonable demands on the patient's patience!

CHAPTER VII.

Aminopolypeptidase.

Secretin tests. The clinical material comprised 10 patients with pernicious anaemia (table 22) and 12 other cases with histamine refractory achylia, as well as 3 cases of hypochylia (where acid could be demonstrated after histamine but not after caffeine) (table 23).

It can be seen from these tables how scattered the results in both groups appear. The material at hand is, however, too small to permit a definite conclusion. Unfortunately the lack of secretin prevented the carrying out of further determinations. In any case, judging from the values obtained, *there exists no striking difference between the two series.* On the contrary the mean values are lying close together (the difference is 0.16 ± 1.6) and so are the values of dispersion (3.44 resp. 3.85).

This is the same result as that first published by HALLÉN and obtained also by ANDERSEN & FABER, although they used secretin only in a few cases to stimulate secretion. It is of course always possible that a clinical material consisting of cases with histamine refractory achylia contains a certain number of potential cases of pernicious anaemia.

Table 22. *Aminopolypeptidase (a.p.p.) [expressed as cu.mm. n/20 HCl used] in gastric juice from cases of pernicious anaemia after stimulation with secretin.*

Case	Age	A.p.p.	Remarks
HJ	64	9.5	untreated case
AA	78	9.3	»
EL	57	2.2	»
AG	69	0	»
AA	71	11.2	treated case
MS	75	8.2	»
VJ	76	5.8	»
JJ	66	6.4	»
GP	72	5.5	»
AJ	81	4.8	»
Mean $\pm$ standard error of the mean		6.29 $\pm$ 1.09	
Standard deviation.....		$\pm$ 3.44	

Table 23. *Aminopolypeptidase (a.p.p.) [expressed as cu.mm. n/20 HCl used] in gastric juice after stimulation with secretin from cases of*

1) Achylia simplex.

2) Hypochylia
(histamine positive).

Case	Age	A.p.p.
GA	73	11.9
EO	46	13.0
GM	58	10.3
SB	60	8.3
OL	45	6.0
HP	49	5.3
EL	59	3.8
AZ	68	3.5
BB	73	2.6
ET	45	2.6
ME	65	1.6
ES	65	4.7
Mean $\pm$ standard error of the mean		6.13 $\pm$ 1.11
Standard deviation		$\pm$ 3.85

Case	Age	A.p.p.
LV	49	2.4
EM	43	2.8
GC	43	4.3

To dispel such a suspicion it is essential that the patients have been kept under observation for several years. We were in a position to do so, and table 24 records 3 of the cases in table 23 which had the lowest values for aminopolypeptidase. When controlled after $4\frac{1}{2}$ years none of them proved to have developed into pernicious anaemia.

Table 24. *Erythrocyte and hæmoglobin values in 3 patients with low figures for aminopolypeptidase at examination $4\frac{1}{2}$ years previously (table 23).*

Case	Time	Hb %	R. b. c. mill.
		100 % = 15.4 gm %	
EL	Febr. '44	90	5.1
	Nov. '48	81	3.8
AZ	March '44	88	5.0
	Nov. '48	84	4.0
ET	March '44	78	3.6
	Nov. '48	72	3.4

Further one can point to the three cases of hypochylia. There is no real cause for regarding them as incipient cases of pernicious anaemia, but they nevertheless show strikingly low values. We are therefore justified in drawing the conclusion that *the determination of aminopolypeptidase with this technique cannot be of any help in the diagnosis of pernicious anaemia.*

Table 25. *Aminopolypeptidase (a.p.p.) [expressed as cu.mm. n/20 HCl used] in 10 ml normal saline instillate (pH 6—7), patient lying on his left side.*

Case	Age	A.p.p.	Remarks
EE	50	1.6	pernicious anaemia
GA	73	1.2	» »
IS	60	3.3	achylia simplex
HP	62	0.5	hypochylia
LEO	74	1.1	»

FOX & CASTLE's already mentioned opinion that in the human stomach intrinsic factor is localised in the fundus-cardia part of the stomach induced us to perform some experiments to find out whether secretin is able to produce secretion of aminopolypeptidase from this part of the stomach. For this purpose the patient was put on his left side, 10 ml normal saline was instilled and left in the stomach during the

hour which the secretin test lasted. Unfortunately only a few of these experiments were completed while secretin was still available. The result will be found in table 25. Even these few experiments seem to indicate that the results thus obtained are not fundamentally different from those given above.

Aminopolypeptidase in gastric mucosa.

Since it was no longer possible to obtain secretin, experiments were taken up to determine aminopolypeptidase in gastric mucosa which was removed at operations for ulcer (gastric or duodenal) and cancer; among the latter cases were but a few patients with pernicious anaemia.

The following *method* was used: as soon as the gastric preparation was made available after the resection small bits of mucosa from the pylorus and glandular mucosa from the fundus were taken (this material was examined and checked microscopically on dyed paraffin section). The material was weighed and immediately frozen with CO₂ and kept fresh in a refrigerator until it was cut into frozen sections of about 100 μ with the microtome. The sections were caught in a phosphate buffer solution with a pH of 7 (the proportion of tissue to buffer was 1 gm/30 ml). Extraction was carried out for one hour by shaking vigorously at room temperature, after which the fluid was drained off. The determination of aminopolypeptidase then took place in the manner described above.

The patients examined included 9 with ulcers (gastric or duodenal), 7 with cancer of the stomach, of these 2 also with pernicious anaemia as well as 1 case with a benign polyp; in all 17 cases (table 26).

It is remarkable that aminopolypeptidase was found in the mucous membrane from both fundus and pylorus not only in the cases of achylia, of which several had an atrophic mucous membrane, but even in the cases of ulcer. With three exceptions the concentration was higher in the mucosa of the pylorus but the difference was not great and not statistically significant (1.04 ± 0.97). Patients with pernicious anaemia showed low values, but two of the other patients with achylia are at the same low level. We therefore need more data, especially of pernicious anaemia, before it will be possible to draw any definite conclusions.

In table 27 all determinations of aminopolypeptidase in the mucosa of the fundus are grouped together with those from the mucosa of the pylorus.

As the result of these investigations about the occurrence of aminopolypeptidase in gastric juice after stimulating with secretin it is only possible to say the following. With the technique here employed no fundamental difference between cases of pernicious anaemia and other achylia has been detected. It is also possible to state that no positive evidence has been obtained to support the view that aminopolypepti-

Table 26. *Aminopolypeptidase (a.p.p.) [expressed as cu.mm. n/20 HCl used] in gastric mucous membrane (removed at operations).*

Case	Age	A.p.p. in fundus gland mucosa	A.p.p. in pylorus gland mucosa	Diagnosis
VJ	45	5.2	8.8	Ulcus ventriculi
GEL	54	5.6	7.9	» »
BG	27	6.9	7.8	» duodeni
EJ	67	6.6	7.4	» »
NE	37	6.0	5.5	» »
ÅS	28	10.7	11.1	» »
GB	20	7.2	9.4	» »
JAL	71	5.1	5.6	» »
FSK	23	4.7	6.5	» »
KAH	61	8.6	8.0	Carcinoma ventriculi
BA	57	11.3	13.3	» »
EL	69	7.3	5.1	» »
OL	74	11.9	12.0	» »
HEA	61	3.8	4.1	» »
KL	59	2.8	5.3	Polypus ventriculi
KED	68	3.1	4.9	{ Anaemia perniciosa
KLK	64	2.3	4.2	{ [+ Carcinoma ventriculi]
Mean $\pm$ standard error of the mean		6.42 $\pm$ 0.70	7.46 $\pm$ 0.67	
Standard deviation ..		$\pm$ 2.89	$\pm$ 2.75	

Table 27. *Aminopolypeptidase (a.p.p.) [expressed as cu.mm. n/20 HCl used] in gastric mucous membrane.*

A.p.p.	Fundus gland mucosa				Pylorus gland mucosa			
	Pernicious anaemia	Achylia simplex	Peptic ulcer	Total	Pernicious anaemia	Achylia simplex	Peptic ulcer	Total
0—5	2	2	3	7	2	3	0	5
6—10	0	2	5	7	0	1	8	9
>10	0	2	1	3	0	2	1	3

dase is identical with intrinsic factor. On the other hand it is impossible to draw the conclusion that aminopolypeptidase is not the intrinsic factor. Several circumstances may be mentioned in this connection: the secretion obtained in the secretin test was extremely viscous and with tube aspiration, which has to be used under clinical conditions, we have to allow for the fact that it is practically impossible to arrive at any correct estimate about the amount of secretion. There are also

too few observations to permit any far-reaching conclusions. Apart from this we should also bear in mind the many unsolved problems which are still to be found in the mechanism of pernicious anaemia in spite of the discoveries of later years.

CHAPTER VIII.

Discussion.

When Castle on the basis of his experiments formulated the theory of extrinsic and intrinsic factors the etiological problem of pernicious anaemia seemed to be solved. Since then, however, the questions regarding the exact mechanism have been much debated. In this place it would be impossible to review the extensive literature on the subject but some special problems will be touched upon.

A question of essential importance is how achylia and pernicious anaemia are mutually related.

There are three possibilities:

1. The achylia is primary and may be looked upon as the cause of the anaemia.
2. The achylia is secondary and caused by the anaemia.
3. Both achylia and anaemia are caused by a third factor.

Naturally, only exceptional cases who later developed pernicious anaemia have had a previous gastric analysis. We are therefore badly informed about the very beginning of the disease. In support of the first possibility many examples may, however, be cited of how achylia has preceded anaemia with shorter or longer periods up to several years. Conceivably this might happen also in alternative 3 if the gastric mucosa is presupposed to be particularly sensitive to the state of deficiency in question.

If the achylia were caused by the anaemia it should be possible to find occasional patients with preserved acid secretion. Such cases are therefore of especial interest in this connection.

It is known that they are rare. ASKEY (1944) has recently collected 47 cases from the literature. Of these he completely rejects 32 on the grounds that they had shown signs of complicating diseases or because the data given did not convince him as to the correctness of the diagnosis pernicious anaemia. Of the remaining 15 cases he considers none to be quite convincing because the examination had not been sufficiently thorough to exclude other diseases (in liver, intestine etc.). It may of course be said of Askey that he is too critical concerning the clinical diagnosis. On the other hand these cases are so important from a theore-

tical point of view that the diagnosis must be completely irrefutable. Their rarity, however, makes it quite plausible that their etiology may be different from the common one. Without doubt achylia is *clinically* still a condition sine qua non for a diagnosis of pernicious anaemia. If acid is present the diagnosis should be regarded with the utmost caution and so must cases when the acid secretion has returned following treatment; such patients are rare indeed.

Anatomical investigations have given somewhat different results.

More recently gastroscopic investigations have been carried out which deserve attention in this connection. It was observed that as a consequence of liver therapy the atrophic changes in the gastric mucosa more or less disappeared (JONES, BENEDICT & HAMPTON 1935, CHEVALIER & MOUTIER 1937, SCHIFF & GOODMAN 1938, SCHINDLER & SERBY 1939 and others) although no restoration of its function could be established. It is, of course, not possible to draw any conclusion about the mucous glands from this gastroscopically observed regeneration of the surface epithelium but it seems possible to conclude *ex juvantibus* that a certain connection exists between the specific state of deficiency and the changes in the gastric mucosa. — Other investigations are, however, less positive in this respect. Thus CAREY (1940) found no effect of liver therapy on the endoscopic appearance of the gastric mucosa in cases of pernicious anaemia. HARDT, SCHWARTZ & STEIGMANN (1948) examined 100 cases of pernicious anaemia gastroscopically and found atrophy of the mucosa in 59%, mostly patchy however, diffuse only in 6 cases. In a further examination carried out after 12—23 months, 6 cases exhibited a normal gastroscopic picture after treatment, but at the same time 4 cases showed atrophy where no pathological changes had been observed earlier. The authors are unable to find a parallel between the clinical picture and the degree of atrophy and assume that there are various factors producing anaemia and epithelial changes in the stomach.

Pathological-anatomical investigations also yielded varying results. Different degrees of atrophy and inflammatory conditions were observed (FABER 1935, MAGNUS & UNGLEY 1938, MEULENGRACHT 1939, COX 1943 and others). No conclusive evidence for a regenerative effect of anti-pernicious treatment could be found. No histological restoration of the glands occurs and the improvement which can be observed gastroscopically may therefore be regarded as purely superficial.

From both the practical and theoretical point of view it would be interesting if some functional test could be developed whereby the achylia of pernicious anaemia could be differentiated from other forms of severe achylia. Among the methods used the neutral red test and pepsin determinations will be discussed first.

As regards the value of the *neutral red* test for this special purpose it may be mentioned that MORRISON (1935—36, 1938) expressed the

opinion that the absence of acid secretion and neutral red excretion in pernicious anaemia cases indicated that the parietal cells also secreted intrinsic factor. Strange to say Bockus (1944) took the same attitude towards the neutral red test, although he maintained that the dye was excreted throughout the stomach, particularly in the antrum-pylorus region which he assumes to be the site of the secretion of intrinsic factor (according to Meulengracht).

The presence of *pepsin* in pernicious achylia has also been the subject of diverse interpretations (cf. p. 190), but on the whole it may be said that according to the literature pepsin is absent in pernicious anaemia or present only in traces.

The investigations of the present author have demonstrated *that* neutral red follows the acid secretion also in cases of achylia. The absence of dye excretion thus indicates the presence of absolute achylia. It may further be found that all the pernicious anaemia cases examined were neutral red refractory. No support was found for the assumption that a more sensitive technique would establish the presence of hypochylia in pernicious anaemia. In 10 cases of pernicious anaemia neutral red was injected together with insulin (16 I.U. intravenously) instead of histamine. But even so no dye excretion could be observed. In addition, it must be emphasized that neutral red refractory achylia exists also without pernicious anaemia. Absence of neutral red in the gastric juice after histamine stimulation is therefore not of crucial importance for the diagnosis of pernicious anaemia.

that the pepsin likewise follows the acid secretion. As was said about neutral red the presence of pepsin in a sample of gastric juice definitely proves that it is not a case of absolute achylia; on the other hand it is not correct to state that the absence of pepsin is a decisive proof of pernicious anaemia.

that in cases of severe achylia, pernicious or not, it is usually possible to release both neutral red and pepsin in acid instillates. If this is interpreted as a sign that both parietal and main cells are still functioning to a certain extent — which seems very plausible — then total atrophy of the fundus glands is uncommon in pernicious anaemia.

Out of our present knowledge it may also be said that neither acid nor pepsin has anything to do with the etiology of pernicious anaemia. There exists no clinically useful method of determining intrinsic factor and, judging the literature, a clear-cut answer cannot be expected when Castle's experiment is carried out in cases of achylia.

The fact that the determination of aminopolypeptidase was taken up for discussion could therefore be both practically and theoretically defended. The authors experiments, unfortunately too few, seem however to indicate that it is not possible in this way to differ between pernicious and simple achylia. Nor do the determinations of aminopolypeptidase carried out on the gastric mucosa of man show any

significant difference between the glandular mucosa of the fundus and the pylorus.

In general it is possible to say that in cases of pernicious anaemia and other real achylia it would seem to be very difficult or impossible to gain sufficiently large amounts of gastric juice for Castle's test under reasonable conditions. The higher secretion volumina from cases of pernicious anaemia which are quoted in the literature (for example HELMER, FOUTS & ZERFAS 190, REIMANN & co-workers 140, GOLDHAMER 160 ml/hr) must have been caused by extra-ventricular admixtures. The volume of gastric secretion is chiefly determined by hydrochloric acid. How the secretion of intrinsic factor behaves quantitatively is unknown. Perhaps its volume is normally small and unchanged even in severe not pernicious achylia but may be so negligible that the secreta are difficult to aspirate with a gastric tube. The highly viscous character of the sample is a further difficulty in this connection. In table 28 the present material is arranged from this point of view, and it will be found that the majority of cases of pernicious anaemia as well as other achylia produced a secretion volume of less than 5 ml/hr.

Table 28. *Volume of gastric juice secreted during one hour after histamine and calculated as volume increase of a 50 ml glycine instillate in cases of pernicious anaemia and simple achylia.*

Volume increase, ml	Number of cases of	
	pernicious anaemia	simple achylia
0	4	2
0—5	15	12
6—10	1	6
11—20	1	1
>20		1

It seems, therefore, possible to accept POLLAND & BLOOMFIELD'S (1935) opinion. They point out that with the insignificant amount of secretion found in severe cases of achylia one cannot expect sufficient amounts of intrinsic factor to be produced. With this in mind they followed 43 cases of uncomplicated achylia for 1—7 years, most of them for 3—4 years, without any of them developing pernicious anaemia. One of them regained the power to produce acid, the others remained anacid. In two of these cases Barnett (vide p. 193) did not succeed in demonstrating intrinsic factor and yet no anaemia developed even after 4 years' control. For this reason Polland and Bloomfield think that another additional factor is needed for anaemia to develop.

By way of comparison we may mention that some of the patients from table 5 (p. 209) were subsequently re-examined especially with a view to discover whether any demonstrable changes in the blood had occurred. Only 7 of them presented themselves for control examinations and the results are to be found in table 29.

Table 29. *Haemoglobin and erythrocyte values in seven patients with neutral red refractory achylia two or three years previously (table 5).*

Case	Time	Hb % 100 % = 15.4 gm %	R. b. c. (mill.)	Remarks
51	April '45	68	3.9	Carcinoma ventriculi, operated
	Sept. '48	75	3.7	
52	Jan. '46	50	3.0	
	Sept. '48	43	3.6	
53	May '45	75	3.5	
	Sept. '48	71	4.1	
57	Oct. '46	82	4.5	
	Sept. '48	71	3.3	
58	April '45	77	3.9	
	Sept. '48	73	4.0	
59	Febr. '46	72	4.4	
	Sept. '48	71	4.0	
60	June '48	84	4.7	Observed since 1943

Particularly interesting are the re-examined cases 57 and 60, the only ones among this material of whom it is known that near relatives suffer from pernicious anaemia (sister and mother respectively). Case 57 had possibly developed pernicious anaemia, though only to a slight degree, but the later reaction to liver therapy seems to support the diagnosis. — Case 60 has been observed longest. Here achylia (histamine and insulin refractory) was diagnosed in 1939 and persisted during various controls, the last one in 1946. In 1943 neutral red test was made and dye was not excreted. In acid instillate the pepsin content was low. Particularly interesting is the fact that the mother suffers from pernicious anaemia and two sisters from recurring iron deficiency anaemia. But the patient himself has no anaemia. — The rest of the re-examined patients showed no sign of anaemia, thus these results agree with those of Pollard and Bloomfield. In connection with case 57 it is pertinent to recall ASKEY's (1944) statement: "although ordinary anacidity per se carries little hazard in relation to the loss of intrinsic factor, it constitutes a definite hazard for an individual in whose family there is pernicious anaemia". In discussing the evidence we should

recall that in cases of pernicious anaemia long spontaneous remissions are often observed (2—3 years and even longer, WALDENSTRÖM 1942).

These observations of grave and chronic achylia without anaemia fit in with cases of total gastrectomy which show no sign of pernicious anaemia after a period of observation of up to 4 $\frac{1}{2}$ years (ASKEY).

CHAPTER IX.

Summary.

For the interpretation of the achylia in a given clinical case it must be important to be able to differentiate them according to severity etc. The literature contains a large number of observations on achylia spuria, i. e. cases showing hydrochloric acid after stimulation with histamine (or insulin). The excretion of neutral red in these cases has never been thoroughly investigated. This question becomes especially important for the discussion of achlorhydria in pernicious anaemia. There are no investigations concerning the differences between achlorhydria in pernicious anaemia and in other conditions e. g. when tested with neutral red. These problems were therefore examined.

This investigation was chiefly based on instillation experiments in the stomach when it was necessary to block the passage between the stomach and the duodenum. This has been possible through a special technique that was described in Chapt. II.

I. Experiments with acid instillation.

If 100 ml 170 millinormal hydrochloric acid is instilled into the closed stomach of normal individuals (with acidity values after histamine ≥ 100 millinormality) there is found a decrease in acidity after one hour of 59 units as the mean value. — If only 50 ml of the same acid were given the mean value for the decrease was 75 units. The difference between the means is significant. — If cases of hypochylia (i. e. < 100 millinormality after histamine) are examined with the same technique with a 100 ml instillate the mean value for acid reduction was 69 units. The difference from the normal cases was significant.

In these experiments no changes in volume that could have been of importance as indicating a dilution were found.

Experiments with simultaneous injection of histamine and atropine or pilocarpine gave no clearcut difference as regards the acidity. The difference that was noted by other authors (Kalk) may well be explained by a dilution with other secretions caused by the pilocarpine. An increased secretion of gastric juice could not be found.

In 5 cases the content of glucosamine was determined in the instillate in order to obtain a measure of the gastric mucus present in the acid. In these experiments there was found no parallelism between decrease in gastric acidity and glucosamine content. No support was thus found for the hypothesis that the mucus might have an influence on the reduction of acidity that was found in these experiments.

2. Neutral red experiments.

In these tests 5 ml of a 1% solution were injected intravenously. Histamine was given subcutaneously at the same time.

The author has shown, with the aid of Teorell's technique for glycine instillations, that excretion of neutral red only occurs when acid secretion may be provoked. The presence of dye in the gastric contents is therefore a sign that acid has been secreted. (Neutral red is also excreted by the liver and in case of bile admixture the occurrence of neutral red is of no importance.) The opinion expressed in the literature that certain patients with true achylia should be able to excrete neutral red is obviously incorrect. In reality such small amounts of acid are secreted in these cases that its presence could not be established with the usual intubation technique. With neutral red it is possible to differentiate these instances of extreme hypochylia from true achylia. In this way the author has been able to show that out of 37 histamine refractory achylia cases (where pernicious anaemia was not present) about one half (19) excreted neutral red and thus were to be regarded as instances of pseudoachylia. A negative neutral red test is therefore not pathognomonic for the achylia of pernicious anaemia even if all examined cases of this malady were neutral red refractory.

In cases of neutral red refractory achylia with or without pernicious anaemia it is possible — with a few exceptions — to provoke excretion of the dye with an acid instillate of sufficient concentration (150 or 170 but not with 120 millinormal). Therefore it seems probable that presence of neutral red indicates secretion of an acid that has a high primary concentration. — A small number of cases were shown to be neutral red refractory even in 170 millinormal acid (5 out of 39 cases with pernicious anaemia and 1 out of 23 other cases).

With the aid of neutral red tests it is then possible to group the histamine refractory achlorhydria cases as follows.

1. Neutral red positive cases excreting the dye after histamine (or insulin) who are in reality to be regarded as instances of severe hypochlorhydria.

The cases who were neutral red refractory at this examination are divided into the following two groups:

2. cases excreting neutral red into an acid instillate and

3. cases who are neutral red refractory even in an acid instillate. This group must be regarded as the most serious form of achlorhydria.

In 18 cases of histamine positive hypochlorhydria a calculation of the primary acidity was made. It was found that the values vary considerably between 141 and 462 millinormality. Many sources of error in the determination of the actual secretion volumes must have strong influence on the exactness of the figures.

3. *Pepsin determinations.*

The determinations have been made in acid instillates from cases of pernicious anaemia and other instances of absolute achylia. The values show great variations in both groups without any significant difference between the groups. It was also evident that measurable quantities of pepsin were secreted in the majority of cases with pernicious anaemia and in this group it has been found such high values as were never observed by previous investigators. This is explained by the technique with acid instillates. It is evident that pepsin is found in 170 millinormal acid instillates even when it is not present in 100 millinormal acid or in glycine solution. The secretion of pepsin seems to depend on the presence of acid. If pepsin is absent in a sample of gastric juice this is usually explained by the fact that *acid* has not been secreted (if the sample has not been standing for some time at alkaline reaction which leads to denaturation). The difference between achlorhydria and achylia obviously disappears and the diagnostic value of pepsin determinations in achylia cases is at the same time more clearly defined and reduced as regards practical importance.

Some cases being neutral red refractory even in 170 millinormal acid still secreted pepsin in this instillate. This may be interpreted as a further indication that the statements in the literature regarding the excretion of neutral red (and secretion of acid) on the one side and secretion of pepsin on the other from different cellular elements may be correct. If this were true the main cells are more resistant than the parietal cells.

4. *Determinations of aminopolypeptidase.*

This enzyme has been shown to be present in gastric contents after secretin stimulation both in cases of pernicious anaemia and in other instances of achylia. No difference was found between these two groups. The values of aminopolypeptidase in the mucosa from the fundus (determined on material from gastric resections) lie very close to the values found in the pyloric mucosa.

It must be pointed out, however, that the relatively low number of observations (caused by shortage of secretin) did not allow a complete discussion of the connection between aminopolypeptidase and intrinsic factor.

Clinical Data.

Hb 100 % = 15.4 gm% haemoglobin.

SeFe = serum iron.

bef. = before treatment.

aft. = after treatment: for serum iron value usually on the 3.—4. day, for platelets generally about 10 days from the beginning of treatment.

Interval refers to the time between first and second value of haemoglobin and erythrocytes.

Numbers (e. g. 158/47) refers to case recors of the Medical Clinic.

[Case 62—73 are used for instillation experiments with acid of varying strength (together with case 30).

Case 74—78 are neutral red negative even in 170 mN acid instillate.]

Case	Hb % Ery. Mill.		Inter- val weeks	SeFe γ%		Platelets 1000		Retic. max. %	Megaloblastic bone-marrow	Remarks
	bef.	aft.		bef.	aft.	bef.	aft.			
19. GH 158/47	26 0.9	51 2.6	2			94	277	27	+	Campolon.
20. AA 2533/44	38 1.0	66 2.9	3	175	42	87	242	32	+	Pernaemon.
21. AA 2508/44	63 2.3	83 3.8	6	254	47	100	257	9	+	Pernaemon forte.
22. K(E)D 2855/45	50 1.7	75 3.9	3			152	236	32	+	Campolon.
23. OJ 604/45	40 1.6	63 4.2	4			140		12	+	Campolon.
24. AW 706/45 pol.	61 2.2	67 2.5	4							Heptomin. Insufficient treatment. After intense tretment in the Medical Clinic a year later Hb 80 Ery 4.5.
25. KAJ 910/46										Diagnosis of pernicious anaemia made 1932 in another hospital. Case record not available.
26. JE 28/45	40 1.5	56 2.5	3	217	31	80	207	28	+	Pernaemon forte.
27. AB 2384/46	46 1.4	56 3.0	3	198	35	250	403	15	+	» »
28. LA 1323/45	27 1.1	61 3.1	3	208	123	85	143	51	+	» »
29. DC 2755/46	62 2.5	84 4.1	3	129	31	261	300	6	+	Folic acid. Insignificant effect of liver therapy. Anaemia perniciosiformis.
30. JA 46/45	67 2.2	80 3.2	4	187	112	150	170	11	+	Pernaemon forte.
31. ES 291/45	38 1.3	62 2.9	3	134	45	56	258	23	+	» »

Clinical Data (Cont.)

Case	Hb % Ery. Mill.		Inter- val weeks	SeFe $\gamma\%$		Platelets 1000		Retic. max. %	Megalo- blastic bone- marrow	Remarks
	bef.	aft.		bef.	aft.	bef.	aft.			
32. SJ 199/46	50 2.2	84 4.9	4			266	357	1.6	+	Campolon.
33. DE 799/45	57 2.0	74 2.9	1			180	555	9	+	Campolon. Pernicious anaemia complicated with myeloid leukaemia.
34. KW 3125/46	53 2.0	86 3.2	3	117	39	285		12	+	Heptomin.
35. EH 959/45	53 2.0	71 3.6	5	94	25	178	207	6	+	Pernaemon forte.
36. K(L)K 2142/37	65 2.1	86 4.4	4			215		5		Campolon.
37. EK 798/45	75 2.8	95 4.1	5	134	58			5	+	Pernaemon.
38. HS 2346/44	25 1.0	70 4.2	12			122	206			Campolon. Insufficient treat- ment.
39. EE 321/48	26 0.8	56 2.1	2	70	40	63	250	30		Campolon.
62. MW 298/45	35 1.5	63 3.3	6	103	32	220	484	19	+	Heptomin.
63. GH 1657/47	40 1.6	68 3.8	4	202	28	88	310	13		Pernaemon forte.
64. HB 1377/45	36 1.2	50 2.5	2	194	44	62	268	25	+	Pernaemon.
65. VL 1127/33	30 1.0	50 2.3	2			228	378	23		Campolon.
66. ES 177/46	36 1.4	63 2.9	6	254	39	86	632	24		Heptomin.
67. HA 821/45	40 1.5	65 3.0	3	123	14	69	280	29	+	Pernaemon forte.
68. MR 353/33	38 1.3	88 3.7	4			112	240	20		Heptomin.
69. II 200/46	28 1.2	56 2.8	2	39	27	125		24	+	"
70. EN 2739/46	60 2.4	88 4.0	6	230	61	205	237	15		"
71. EP 510/49	31 1.4	43 2.7	3	123	26	64	174	12		Campolon.
72. SM 353/46	35 1.3	45 3.5	2	182	36	135		35		Heptomin.
73. HB 729/49	57 2.5	82 5.1	6	99	35	100	141	15	+	Campolon.
74. GE 1161/45	40 1.5	72 3.6	3	290	65	75	112	28	+	Heptomin.

Clinical Data (Cont.)

Case	Hb % Ery. Mill.		Inter- val weeks	SeFe $\gamma\%$		Platelets 1000		Retic. max. %	Megalo- blastic bone- marrow	Remarks
	bef.	aft.		bef.	aft.	bef.	aft.			
75. KEK 976/45	32 1.2	62 2.8	3	211	47	89	239	37	+	Pernaemon forte.
76. EL 241/45	55 1.6	75 3.5	3	215	37	154		8	+	» » (Table 21).
77. KH 2218/44	62 2.4	83 3.4	3	119	63	214	161	1.8	+	» »
78. EL 312/49	43 1.9	67 4.0	4	185	39	181	182	25	+	Heptomin (Table 20).
HJ 250/44	50 1.6	74 3.0	3	112	47	63	111	11	+	Pernaemon forte.
EL 669/44	84 3.4	82 3.9	2			132	276	1.0		Campolon. 1941, 43 and 44 glossitis with atrophic lingual papillae; every time liver therapy with excellent effect. (Table 23).
AG 2615/46	43 1.4	73 2.8	4	193	52	67	400	15	+	Heptomin.
AA 154/45	66 2.6	81 3.4	5	140	35		463	4	-	Campolon.
MS 828/24	43 1.7	54 2.8	4	69	26	134	525	13	+	Pernaemon forte.
VJ 1192/45	43 1.4	68 3.3	3	225	36	84	366	10	+	» »
JJ 860/44	52 1.4	63 2.6	2	195	30	94	500	17	+	» »
GP 696/44	82 2.9	94 4.1	4	235	33	85	173	6	+	»
AJ 1918/46	60 2.2	54 3.6	4	178	43	204		8		Heptomin, Fe.
EF 926/48	38 1.8	74 3.6	4	225	46	76	392	39	+	Folic acid.
MG 1920/48	25 1.4	60 2.8	3			54	428	27	+	Campolon.
IP 812/48	31 1.5	53 2.9	5	194	30			16		Heptomin.
JH 715/48	42 1.1	72 3.8	2	255	34	98		28		»
JG 801/48	45 1.9	67 3.5	4	194	45	120	341	11	+	»
EÖ 998/48	45 1.5	64 2.7	2	85	31	121	485	19	+	Campolon.
HÖ 1276/44	46 1.8	70 3.3	3	130	29	143	220	13	+	Pernaemon forte.
EV 2512/43	38 1.3	56 2.9	4			103	250	17		Campolon.

References.

- ABBOTT, W. O. *New Eng. J. Med.* 225: 641, 1941.
- ALLEY, A. quoted from IHRE.
- ALVAREZ, W. C. *Gastroenterology* 1: 534, 1943.
- ANDERSEN, B. C. r. d. trav. du Laborat. Carlsberg, ser. chim. 22: 36, 1938.
- ANDERSEN, J. and FABER, M. *Nord. Med.* 30: 1379, 1946.
- ASKEY, J. M. *Ann. Int. Med.* 2: 1, 1944.
- BABKIN, B. P. *Secretory Mechanism of the Digestive Glands*. New York 1944.
- BARNETT, CH. W. *Am. J. Med. Sci.* 182: 170, 1931.
- *Am. J. Med. Sci.* 184: 24, 1932.
- BECKER, K. P. *Deutsch. Arch. klin. Med.* 176: 669, 1934.
- BLOOMFIELD, A. L. and POLLAND, W. S. *J. Clin. Inv.* 14: 321, 1935.
- BOAS, J. *Diagnostik und Therapie der Magenkrankheiten*. Leipzig 1925.
- BOCKUS, H. L. *Gastroenterology* vol. I. Philadelphia 1944.
- BOLDYREFF, W. N. *Erg. Physiol.* 11: 121, 1911.
- *Acta Med. Scand.* 82: 111, 1934.
- BOLTON, C. and GOODHART, G. W. *J. Physiol.* 73: 115, 1931.
- BOWIE, D. J. and VINEBERG, A. M. quoted from BABKIN.
- BRAITHWAITE, L. R. *Brit. J. Surg.* 31: 3, 1943—44.
- BULL, H. B. and GRAY, J. S. *Gastroenterology* 4: 175, 1945.
- CAREY, J. B. *Am. J. Dig. Dis.* 7: 160, 1940.
- CASTLE, W. B. *Am. J. Med. Sci.* 178: 748, 1929.
- CASTLE, W. B., HEATH, C. W., and STRAUSS, M. B. *Am. J. Med. Sci.* 182: 741, 1931.
- CASTLE, W. B., and MINOT, G. R. *Pathological Physiology and Clinical Description of the Anemias*. New York 1936.
- CASTLE, W. B., TOWNSEND, W. C., and HEATH, C. W. *J. Clin. Inv.* 9: 2, 1930.
- *Am. J. Med. Sci.* 180: 205, 1930.
- CHANG, H. C., YANG, C. S., and KEEFER, C. S. *Nat. Med. J. China* 15: 752, 1929.
- CHEVALLIER, P., and MOUTIER, F. *Sang* 11: 935, 1937.
- COX, A. J., *Am. J. Pathol.* 19: 491, 1943.
- DAIKHOVSKY, J. I., and SOLOVEI, M. G. *Acta Med. Scand.* 83: 79, 1934.
- DAVENPORT, H. *Gastroenterology* 1: 4, 1943.
- DAVIDSON, P. B., WILLCOX, E., and HAAGENSEN, C. D. *J. A. M. A.* 85: 794, 1925.
- DAVIES, D. T. *Quart. J. Med.* 24: 447, 1930—31.
- DAWSON, A. B. *Am. J. Roentgen.* 13: 320, 1925.
- DAWSON, A. B., and IVY, A. C. *Am. J. Physiol.* 73: 304, 1925.
- EINHORN, M. *Die Duodenalsonde*. Leipzig 1924.
- ELLIOT, A., RISHOLM, L., and ÖBRINK, K. J. *Acta Med. Scand.* 60: 267, 1942.
- ENOCKSSON, B., and KARLSSON, S. *Acta Med. Scand. Suppl.* 78: 386, 1935.
- FABER, K. *Gastritis and its Consequences*. Copenhagen 1935.
- FABER, K., and HOLST, J. E. *Acta Med. Scand.* 69: 46, 1928.
- FOX, J. H., and CASTLE, W. B. *Am. J. Med. Sci.* 203: 18, 1942.
- FRANKEL, E. *Am. J. Dig. Dis.* 12: 15, 1945.
- FULD, E. *Münch. Med. Wschr.* 55 (2): 2264, 1908.
- GILLMAN, TH. *South. Afr. J. Med. Sci.* 8: 50, 1943.
- *Gastroenterology* 3: 188, 1944.
- GLAESSNER, K., and WITTGENSTEIN, H. *Klin. Wschr.* 2: 1650, 1923.
- (Hamperl, H.) *Arch. Verd.krankh.* 34: 303, 1925.
- GOLDHAMER, S. M. *Am. J. Med. Sci.* 191: 405, 1936.
- *Am. J. Med. Sci.* 193: 23, 1937.
- GOLDHAMER, S. M., ISAACS, R., and STURGIS, C. C. *Am. J. Med. Sci.* 188: 193, 1934.
- GRANT, RH. *Am. J. Physiol.* 132: 467, 1941.
- *Am. J. Physiol.* 135: 496, 1941—42.
- GRAY, J. S. *Gastroenterology* 1: 4, 1943.

- HALLÉN, L. Nord. Med. 20: 2363, 1943.
— Acta Med. Scand. Suppl. 90: 398, 1938.
— Nord. Med. 25: 225, 1945.
- HAMPERL, H., vide GLAESSNER and WITTGENSTEIN.
- HARDT, L. L., SCHWARTZ, S. O., and STEIGMANN, F. Gastroenterology 10: 108, 1948.
- HARTFALL, S. J., and WITTS, L. J. Guy's Hosp. Rep. 83: 24, 1933.
- HEIDENHAIN, R. quoted from KATSCH.
- HELMER, O. M., FOUTS, P. J., and ZERFAS, L. G. J. Clin. Inv. 11: 1129, 1932.
- HENNING, N., Arch. exper. Pathol. Pharmacol. 165: 191, 1932.
— Die Entzündung des Magens. Leipzig 1934.
- HENNING, N., and JÜRGENS, R. Münch. Med. Wschr. 77: 1961, 1930.
- HERRIOTT, R. M. J. Gen. Physiol. 22: 65, 1938—39.
- HIRABAYASHI, K. quoted from MORRISON, GARDNER and REEVE .
- HOLLANDER, F. J. Biol. Chem. 97: 585, 1932.
— J. Biol. Chem. 125: 161, 1938.
— Gastroenterology 1: 4, 1943.
- HORSTMANN, P. Nord. Med. 4: 3566, 1939.
- HUEPER, W. C., and DE CARVAJAL-FORERO, J. Am. J. Roentgenol. 52: 529, 1944.
- IHRE, B. Acta Med. Scand. Suppl. 95, 1938.
— Nord. Med. 5: 374, 1940.
— Nord. Med. 10: 2036, 1941.
— Nord. Med. 33: 388, 1947.
- INGRAHAM, R. C., and VISSCHER, M. B. J. Gen. Physiol. 18: 695, 1934—35.
- IVY, A. C. quoted from IHRE.
- JONES, C. M., BENEDICT, E. B., and HAMPTON, A. O. Am. J. Med. Sci. 190: 596, 1935.
- KAIJSER, K. Magsaftundersökningar på barn. Stockholm 1943.
- KALK, H. Das Geschwür des Magens und Zwölffingerdarmes. Berlin 1931.
— Klin. Wschr. 11: 270, 1932.
- KALK, H., and BONIS, A. Deutsch. Arch. klin. Med. 173: 53, 1932.
- KATSCH, G. Krankheiten des Magens. Von Bergmanns-Staehelins-Salles Handb. d. inn. Med. 3. Berlin 1938.
- KATSCH, G., and KALK, H. Klin. Wschr. 1: 1119, 1926.
- KEEFER, C. S., and BLOOMFIELD, A. L. Bull. Johns Hopk. Hosp. 39: 304, 1926.
- KEMPER, W. Arch. Verd. krankh. 47: 87, 1930.
- KESAVALU, A., and MANN, F. C. Surgery 14: 578, 1943.
- KIRSNER, J. B., NUTTER, P. B. and PALMER, W. L. J. Clin. Inv. 19: 619 1940.
- KOBAYASHI, K. Acta schol. med. Univ. Kyoto 8: 465, 1925—26.
- KOLM, R., KOMAROV, S. A., and SHAY, H. Gastroenterology 5: 303, 1945.
- KONJETZNY, E. G. Henkes und Lubarschs Handb. d. spez. path. Anat. 4. Berlin 1928.
- LANGLEY, J. N. quoted from BABKIN.
- LANGLEY, J. N. and EDKINS, J. S. quoted from BABKIN.
- LERNER, H. H., ASHER, L., and ANDREWS, K. Am. J. Dig. Dis. Nutr. 9: 109, 1942.
- LINDE, S., TEORELL, T., and ÖBRINK, K. J. Acta Physiol. Scand. 14: 220, 1947.
- LINDERSTRÖM-LANG, K., HOLTER, H., and OHLSEN, A. quoted from BABKIN.
- LIU, A. C., YUAN, I. C., and LIM, R. K. S. quoted from BABKIN.
- LUNDHOLM, I. Hereditary Hypochromic Anemia. Upsala 1939.
- LURIA, R. A., and MIKRIN, A. I. Arch. Verd.krankh. 32: 325, 1925.
- MACLEAN, H., and GRIFFITHS, W. J. J. Physiol. 66: 356, 1928.
- MARUNO, Y. in MATSUO, I. Biol. Untersuch. über Farbstoffe 2: 520, 1934—35.
- MEULENGRACHT, E. Acta Med. Scand. 82: 352, 1934.
— Acta Med. Scand. 82: 375, 1934.
— Acta Med. Scand. 82: 384, 1934.
— Acta Med. Scand. 85: 50, 1935.
— Acta Med. Scand. 85: 79, 1935.
— Nord. Med. 1: 11, 1939.
- MESCHÉDE, K. Arch. Verd.krankh. 61: 318, 1937.

- MILLER, T. G., and ABBOTT, W. O. *Am. J. Med. Sci.* 187: 595, 1934.
- MINOT, G. R., and CASTLE, W. B. *Lancet* 113: 319, 1935.
- MINOT, G. R., and MURPHY, W. P. *J. A. M. A.* 87: 470, 1926.
- MORRISON, S. J. *Lab. Clin. Med.* 21: 827, 1935—36.
- *New Internat. Clin.* 1: 119, 1938.
- *Am. J. Dig. Dis.* 5: 617, 1938.
- MORRISON, S., GARDNER, R. E., and REEVES, D. L. *J. Lab. Clin. Med.* 21: 822, 1936.
- MÄNNLE, E. *Deutsch. Med. Wschr.* 62: 1336, 1936.
- NORDENFELDT, P. J., and TEORELL, T. *Acta Med. Scand.* 85: 525, 1935.
- NORTHROP, J. H. quoted from BABKIN.
- ODIN, M. En socialhygienisk undersökning i Västerbottens och Norrbottens län. Lund 1934.
- OSTERBERG, A. E., VANZANT, F. R., ALVAREZ, W. C., and RIVERS, A. B. *Am. J. Dig. Dis. Nutr.* 3: 35, 1936.
- PALMER, W. L., KIRSNER, J. B., and NUTTER, P. B. *Am. J. Dig. Dis.* 7: 427, 1940.
- PAVLOV, I. P. quoted from BABKIN.
- PIERSOL, G. M., BOCKUS, H. L., and BANK, J. *Am. J. Med. Sci.* 170: 405, 1925.
- PLENK, H. quoted from BABKIN.
- POLLAND, W. S. *J. Clin. Inv.* 9: 319, 1930—31.
- POLLAND, W. S., and BLOOMFIELD, A. L. *J. Clin. Inv.* 7: 45, 1929.
- *J. Clin. Inv.* 7: 57, 1929.
- *J. Clin. Inv.* 9: 107, 1930—31.
- REIMANN, F., and FRITSCH, F. *Z. klin. Med.* 126: 469, 1933—34.
- REIMANN, F., SALUS, F., and WEIL, R. *Z. klin. Med.* 126: 579, 1933—34.
- REIMANN, F., and WEIL, R. *Z. klin. Med.* 126: 568, 1933—34.
- RICHTER, O., IVY, A., and KIM, M. S. quoted from WHITBY and BRITTON.
- RIEGEL, E. quoted from IHRE.
- RUFFIN, J. M., and DICK, M. *Ann. Int. Med.* 12: 1940, 1939.
- SALUS, F., and REIMANN, F. *Klin. Wschr.* 13: 986, 1933.
- SCHIFF, L., and GOODMAN, S. (1938) quoted from SCHINDLER.
- SCHIFF, L., and GOODMAN, S. *Am. J. Dig. Dis. Nutr.* 7: 14, 1940.
- SCHINDLER, R. *Gastroenterology* 1: 532, 1943.
- *Gastritis*. New York 1947.
- SCHINDLER, R., KIRSNER, J. B., and PALMER, W. L. *Arch. Int. Med.* 65: 78, 1940.
- SCHINDLER, R., NUTTER, P. B., GROOM, H. E., and PALMER, W. L. *Arch. Int. Med.* 66: 1060, 1940.
- SCHINDLER, R., and SERBY, A. M. *Arch. Int. Med.* 63: 334, 1939.
- SHAY, H., GERSHON-COHEN, J., FELS, S., and MUNRO, F. L. *Am. J. Dig. Dis.* 6: 535, 1939.
- SHAY, H., KATZ, A. B., and SCHLOSS, E. M. *Arch. Int. Med.* 50: 605, 1932.
- SJÖGREN, B. *Acta Med. Scand.* 106: 479, 1941.
- TEORELL, T. *Skand. Arch. Physiol.* 66: 225, 1933.
- *Acta Med. Scand.* 85: 518, 1935.
- *J. Gen. Physiol.* 23: 263, 1939.
- *J. Physiol.* 97: 308, 1940.
- *Gastroenterology* 9: 425, 1947.
- VAHLQUIST, B. *Das Serumseisen*. *Acta Pediatr.* vol. XXVIII Suppl. V, 1942.
- WALDENSTRÖM, J. *Nord. Med.* 16: 3208, 1942.
- WARREN, R. *Proc. Soc. Exp. Biol. Med.* 41: 287, 1939.
- WARREN, R., KARR, W. G., HOFFMAN, O. D., and ABBOTT, W. O. *Am. J. Med. Sci.* 200: 639, 1940.
- WELIN, G., and FRISK, R. *Acta Med. Scand.* 90: 543, 1936.
- WHITBY, L. E. H., and BRITTON, C. J. C. *Disorders of the Blood*. London 1942.
- WILKINSON, J. F., and KLEIN, L. *Quart. J. Med.* 3: 341, 1934.
- WINKELSTEIN, A. *Am. J. Med. Sci.* 203: 419, 1942.
- WINKELSTEIN, A., and MARCUS, J. M. *J. A. M. A.* 92: 1238, 1929.
- WOLF, S., and WOLFF, H. G. *Human Gastric Function*. New York 1943.

ZIMMERMANN, K. W. quoted from KATSCH.

ÅGREN, G. *Enzymologia* 10: 161, 1942.

— *Arkiv för Kemi, Mineralogi o. Geologi* 16 B, n:o 6, 1942.

— *Arkiv för Kemi, Mineralogi o. Geologi* 17 B, n:o 16, 1943.

— *Nature* 154: 430, 1944.

ÅGREN, G., and LAGERLÖF, H. *Acta Med. Scand.* 90: 1, 1936.

ÅGREN, G., and WALDENSTRÖM, J. *Acta Med. Scand.* 119: 167, 1944.

— *Acta Med. Scand.* 196: 432, 1947.

ÖBRINK, K. J. *Acta Physiol. Scand.* vol. 15, suppl. 51, 1948.

UPPSALA 1949. ALMQVIST & WIKSELLS BOKTRYCKERI AB 493513